APPLICATION OF RESPONSE SURFACE METHODOLOGY (RSM) FOR THE PRODUCTION AND OPTIMIZATION OF EXTRUSION PARAMETERS ON EXTRUDED SNACKS FROM THE BLENDS OF AFRICAN YAM BEAN, SORGHUM AND MORINGA LEAF POWDER

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Abstract
Snacks from lesser known legumes of African yam bean and Moringa leaf powder fractions blended with sorghum to enhance protein quantity and quality was developed in this study through extrusion cooking technology. A five-level-three-factor central composite rotatable design (CCRD) was adopted for the formulation and optimization of the process variables.The main objective was to obtain the optimum level of Feed composition (X_1), feed moisture level (X_2) and exit barrel temperature (X_3) that will produce optimum snack having appreciably high protein and lysine content. ANOVA indicated significance ($p<0.05$) of the models fitted in describing the relationship between the input and output variables in its natural state. The coefficient of the determination was also greater than 50% and non-significant lack-of-fit test. Numerical optimization results indicated that the optimum input variables were 120°C barrel temperature, 42.38g/100g feed composition and 22.89% feed moisture composition, 26.65g/100g protein, 68.99%carbohydrate and lysine of 4.11mg/g protein. These data will sharpen the snacks food industry by providing wide opportunity for new food production using sorghum and lesser known legumes, thereby increasing the economic values of both lesser known legumes such as African yam bean and Moringa leaf. Also the data obtained from the study can also be used for the control of product characteristics and possible projection for the commercial production of extruded snack or any enriched protein based food from the blends of African yam bean, sorghum and Moringa leaf.

Keywords: African yam bean, sorghum, Moringa leaf powder, Response Surface Methodology, Optimization, extruded snack.

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1. INTRODUCTION

A snack is a portion of food often smaller than a regular meal, generally eaten between meals. In developed countries, snacks are eaten in between meals to check hunger, provide energy and tasty appeal; while in developing countries, they are eaten as a main meal because of their ready availability and affordability.Snacks, however, are high in calorie and fat and low in proteins, vitamins and micronutrients (Ranhotra and Vetter, 1991).
Cereals and legumes play important role in the diets of many people in Africa and Asia, and are the major sources of proteins, calories, vitamins and minerals (Nkama *et al*.,2009). The functionality of these components in the different cereals determines, to a large extent, their uses as food and industrial raw material. There has been wide advocacy for cereal/legume blending in the face of deepening problems of hunger and malnutrition in the least developed countries. Several reports have shown that complementation could be achieved by mixing legumes and cereals which abound in the tropics (Anuonye *et al*., 2007; 2009). Even though cereals are deficient in lysine, they have sufficient amounts of sulphur-containing amino acids which are limiting factors in legumes (Enwere, 1998).
The increasing inclusion of legumes in the manufacturing of various functional food products is due to their high protein, energy and micronutrient content (Asgar *et al*., 2010).

The development of such product is the key to improving nutrition for the malnourished, the under-5s and pregnant women through an inexpensive cereal- legume based product.
African yam bean (AYB) is one of the lesser known legumes (Apata and Ologhobo, 1990) and widely cultivated in the southern parts of Nigeria. AYB is rich in lysine but deficient in sulphur amino acids.
In Nigeria and indeed in many African countries, sorghum has been used in the formulation of ready-to-eat cereal products. Akpapunam and Darbe,(1994) noted that it is possible to improve the nutritional quality of cereal proteins by combination with cheaper and more available plant protein sources such as legume to form part of the infant and adult snack food.
Moringa oleifera is an example of the underutilized crops that has great potentials. The plant *Moringa oleifera* is a small fast growing tree found in the tropical regions. They are used for soup preparation especially in the dry season when there is scarcity of other more popular vegetables. Moringa leaves had been known to combat malnutrition in infants and nursing mothers. Moringa leaves have been reported to be rich in nutrient with dry leaves containing as much as 30% protein. The leaves are sources of the sulphur-containing amino acid such as methionine and cystine which are often in short supply in most legumes (Martin *et al*., 1998). The amino acid balance and micronutrient content is also very good.
Extrusion cooking technology is a continuous hightemperature short time (HTST) food processing techniques,in which mechanical energy is combined with heat energy togelatinize starch and denature proteins, plasticizing andreorganizing food materials to create new shaped andtextured products, and also has the ability to inactivateenzymes, destroy some toxic substances and reducemicrobial activity (Ding *et al*., 2005). It has been used in the cerealindustry for several years to produce many foods and foodingredients such as breakfast cereals, snack foods, babyfoods, pasta products, extruded bread, modified starches,beverages, powders, meat and cheese analogues, texturedvegetable protein, and blended foods such as corn starch andgrounded meats (Rhee *etal*., 1999). It is a technology with high versatility andefficiency, low cost, high output per unit time and shortreaction time, with relatively no waste generated (Nabeshimaand Grossmann, 2001). During extrusion process, chemicalmodifications and structural changes occurs in the rawmaterials, such as starch gelatinization (Vanden Einde *et al*., 2004), protein denaturation (Iwe *et al*.,2004), pigment and vitamin degradation (Ilo and Berghofer,1999), and loss of volatile compounds (Bhandani *et al*.,2001). These changes resulted in new food product with newfunctional, nutritional and sensory qualities (Bryant, *et al*.,2001). The knowledge of changes in extruder operatingvariables therefore provide necessary information for theprediction of what fraction of food materials will undergospecific reaction during extrusion process and its possibleeffects on the quality of finished product. Ding *et al*., (2005)reported that little change in extrusion variables such as feedcompositions, feed moisture content, screw speed, screwgeometry, die configuration; feed rate, processingtemperature could greatly affect finished product quality.This therefore has placed a critical need on food scientists toproperly and effectively optimize production variables ifextrusion technology is to be adopted.Response surface methodology (RSM) and centralcomposite design (CCD) provides an ideal tool forinvestigating and optimizing process and product parametersin food processing. RSM is a collection of mathematical andstatistical techniques that are useful for modeling andanalyzing of problems in which a response of interest isinfluenced by several variables and the objective is tooptimize this response (Noordin *et al*.,2004). Several workers (Filli *et al*.,2011 and 2012) have used RSM to predict optimum

foodprocessing conditions. Optimal process and product designwill further improve efficient utilization of broken rice in theproduction of high quality nutritious products, reduceproduction cost, and hasten the upscale of these productsfrom pilot status to industrial scale and also facilitate easytroubleshooting and quality control at large scale.

Optimization of extrusion cooking therefore, may involve critical consideration of process parameter, system parameterand product quality.

The current study focuses on the optimization of extrusionparameters in the use of African yam bean, sorghum and Moringa leaf powder for theproduction of extruded snacks using responsesurface methodology and central composite design.

2.MATERIAL & METHODS

2.1.Flour preparation from African yam bean

Five kilograms of the AYB seeds were sorted, washed and sun dried. The AYB seeds were roasted for 10min, milled (attrition mill) and sieved into fine flour with 1mm mesh sieve.

2.2. Flour preparation from sorghum

The sorghum flour was prepared using the method of Murty and House, (1980). Five kilograms of sorghum seed were sorted, cleaned, dehulled, winnowed, milled (attrition mill), sieved into fine flour to remove the dust and contaminants.

2.3. Preparation of Moringa leaf powder

Moringa leaves were washed and dried at room temperature. The dried leaves were milled into powder and sieved with sieve of 0.3mm aperture size to get Moringa powder.

2.4. Blend Preparations and Moisture Adjustment

Sorghum flour (SF) and African yam bean flourwere mixed at various weight ratios, and the total moisture contents of the blends adjusted to the desired values with a mixer as described by Zasypkin and Tung-Ching Lee, (1998). Weights of the components mixed were calculated using the following formula:

$$C_{SF} = \frac{r_{SF} \times M \times (100-w)}{100 \times (100- w_{SF})} \quad (1)$$

$$C_{AF} = \frac{r_{AF} \times M \times (100-w)}{100 \times (100- w_{AF})} \quad (2)$$

$$W_X = M - C_{SF} - C_{AF} \quad (3)$$

Where C_{SF} and C_{AF} are the masses of sorghum flour and African yam bean flour respectively, r_{SF} and r_{AF} are the respective percentages of sorghum and African yam bean flour in the blend, ($r_{SF} + r_{AF} = 100\%$); where M is the total mass of the blend; w, the moisture content of the final blend, percentage wet weight basis (w.w.b); W_X is the weight of water added; and W_{SF} and W_{AF} are the moisture contents of S_F and A_F respectively. One percentage Moringa leaf powder was added to each formulation;and the samples were put in closed plastic buckets and stored overnight (32±2°C).The feed materials were then allowed to stand for 3hrs toequilibrate at room temperature prior to extrusion exercise.

2.5. Extrusion Cooking Processing

Extrusion cooking was performed in a locally fabricated FST 001 single screw extruder at the Department of Food Science and Technology, University of Nigeria, Nsukka. The extruder was fed manually through a conical shaped hopper mounted vertically above the end of the extruder. The die has 0.4cm diameter and the variables considered were feed composition, feed moisture content and barrel exit temperature. The barrel temperature ranges were between 100-140^{0}C and feed moisture content was set at 20-30%.

2.6. Experimental Design and Statistical Analysis

Table 1. Independent variables and natural levels used for Central Composite Rotatable Design.

Variables	Coded variables -α	Low	Medium	High	+α
	-1.682	-1	0	1	1.682
Feed composition (X_1)	3.4095	15	32	50	60.5905
Feed Moisture content (X_2)	16.5910	20	25	30	33.4090
Barrel temperature (X_3)	86.364	100	120	140	153.636

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 α = (2n)1/4 where n is the number of variables.

Table 2: Experimental design for extrusion in **their coded forms and natural units**

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	15	20	100
2	1	-1	-1	50	20	100
3	-1	1	-1	15	20	140
4	1	1	-1	50	20	140
5	-1	-1	1	15	30	100
6	1	-1	1	50	30	100
7	-1	1	1	15	30	140
8	1	1	1	50	30	140
9	-1.682	0	0	3.4095	25	120
10	1.682	0	0	60.5905	25	120
11	0	-1.682	0	32	25	86.36
12	0	1.682	0	32	25	153.64
13	0	0	-1.682	32	16.5910	120
14	0	0	1.682	32	33.4090	120
15	0	0	0	32	25	120

-1=lowest value, 1=Highest value,0=medium value, -1=-α, 1=+α, feed composition (X_1), barrel exit temperature (X_3), feed moisture content (X_2). Each design point was in triplicate and the average recorded. The experimental runs were randomized.

The extrusion conditions were optimized with a threefactorfive-level central composite rotatable design (CCRD)(Box and Hunter, 1957). Response Surface Methodology(RSM) was used to investigate the effect of the independentvariables on the responses. Feed composition (X1),feed moisture content (X2) and extruder barrel temperature (X3) were the independent variables consideredand the qualities of the finished products were the responsevariables measured. In order to objectively define theexperimental ranges, preliminary experiments wereconducted to establish the narrower, more effective ranges ofthe independent variables (X1, X2, and X3) prior to theexperimental runs. As the design value ranges wereestablished, they were coded to lie at ±1α for the factorialpoints, 0 for the center points and ±1α for axial points. Thecodes were calculated as a function of the range of interest ofeach factor as presented in Table 1. The experiments wererandomized to maximize the effects of unexplainedvariability in the observed responses due to extraneousfactors, while five replicates at the center of the design wereused to allow for estimation of pure error sum of square andlack-of-fit. Analysis of variance (ANOVA) was conducted todetermine significant differences among the mean treatmentcombinations.

2.7. Process Optimization

A second order polynomial regression equation wasmodeled on the basis of the experimental data and optimumparameters defined using Matrix Laboratory (MATLAB14.13) Software. From the resulting values, for each of theresponse variable, the coefficients of the polynomial equation(βo, βi and βij) are determined and the equation simplifiedbased on the influence of the factors on the final response. The responses were then expressed as second-orderpolynomial equation according to Eq. 4.

$$Y=f(y) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 \sum_{i=1}^{k} \quad \sum_{i=1}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (4)$$

Where Y is the predicted response used as a dependentvariable, k is the number of independent variables consideredin the experiment; βo constant coefficient and βi, βij and βii arethe coefficient of linear, interaction and square termsrespectively, while ε is the random error term. Multivariateregression analysis with model equation (4) was carried outon the data using MINITAB 14.13 statistical software(Manitab Inc. USA) to yield equation 5 which was used to
optimize the product responses (Filli *et al*, 2011).

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X^2{}_1 + \beta_{22} X^2{}_2 + \beta_{33} X^2{}_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon \quad (5)$$

The outline of the experiments with the coded and naturalvalues are presented in Table 2

2.8. Validation of Fitted Models

To check if the fitted models provide an adequateapproximation to the real system, it is often important tocheck model adequacy. Unless a model shows adequate fit,proceeding with optimization may lead to misleading results.In this study, numerical validations were conducted on thefitted models. Numerical methods involve the analysis of thecoefficient of determination (R^2) and adjusted coefficient ofdetermination (R^2adj) calculated as:

$$R^2 = 1 - \frac{ss\ residual}{ss\ model + ss\ residual} \quad (6)$$

$$\text{Adjusted } R^2 = 1 - \frac{n-1}{n-p}(1-R^2) \quad (7)$$

Where *SS* is the sum of squares, n is the number ofexperiments and p is the number of predictors in the modelnot counting the constant term. R^2 value close to unity andR^2_{adj} close to R^2 ensure satisfactory fitting of the model tothe real system. Probability value (p-value) was also used tocheck for the significance of each factor and interactionbetween the factors. The smaller the p-value, the moresignificant is the corresponding coefficients (Mason *et al.*2003). Since triplicate measurements were recorded duringanalysis of the response variables, a lack-of-fit test wasconducted to examine the significance of replicate error incomparison to the model dependent error. This test split theresidual or error sum of squares into two parts, one due topure error as a result of duplicate measurement and thesecond due to lack-of-fit which is the ratio between thelack-of-fit mean square and pure error mean square. F-test can then be used to measure wither the lack-of-fit isstatistically significant or not at the described level ofprobability. Non-significant lack-of-fit was considereddesirable.

2.9. Proximate Composition

Protein, fat, ash and dietary fiberwere determined according to AOAC method (2010). Thepercentage carbohydrate was calculated by difference.

2.10. Amino Acids Profile Analysis

Amino acids were determined according to methodsdescribed by Onyeike *et al.* (2005) as modified by Anyalogbu*et al.*(2015). Five (5g) of sample was weighed into extractionthimble of Soxhlet extraction apparatus and 60ml ofchloroform/methanol (2:1 v/v) for extraction. This wasfollowed by hydrolysis by weighing the defatted sample intoa glass ampoule and

7ml of 6MHCl added. The glassampoule was then sealed with flame from Bunsen burnerbefore incubation at 105±2°C for 22hr to effect hydrolysis(Nwonsu, *et al*., 2008). After incubation, the ampoule wasallowed to cool to ambient temperature (32±2°C) beforeopening at the tip and the content filtered through filter paperto remove humis. The protein hydrolysate was evaporated todryness at 40°C under vacuum in a rotary evaporator (BuchiRotavopour Switzerland) and the residual dissolved with 5mlacetate buffer (pH 2.0), in a specimen bottle and stored in afreezer for analysis. 10ml of the dissolved residual wascollected with a micro-syringe and dispensed into thecartridge of the TSM amino acid analyser. The amino acidswere separated on the ion-exchange column through acombination of change in pH and cation strength. The postcolumn reaction between ninhydrin and amino acids elutedfrom the column formed Riemann's purple, adiketohydrinhylidene-diketohydrindamine (Friedman, 2004).The reaction was therefore monitored at 440nm and 570nmwavelengths. It took about 1hr, 15 minutes to complete thereaction. Net height of each peak produced by the chartrecorder of TSM (each representing an amino) wasmeasured. The half-height of the peak on the chart wasfound and width of the peak on the half height wasaccurately measured and recorded. Approximately area ofeach peak was then obtained by multiplying the height withthe width at half-height. Norleucine was added as internalstandard and a standard amino acid mixture (Beckman No.338088, Beckman Coulter, CA) was measured under thesame condition as sample. Finally, the amount of eachamino acid present in the sample was calculated as g/100gprotein. The amino acids, leucine, isoleucine, lysine,methionine, phenylalanine, threonine, tryptophan, valine tyrosine and tryptophan were determined.

3. RESULTS AND DISCUSSION

3.1. Model Fitting and Validation

This study was carried out to produce nutritious extrudedsnack and to optimize the productionprocess variables and product quality using response surfacemethodology and central composite design. Process variablesconsidered were feed composition (X_1), feed moisture content(X_2) and barrel temperature (X_3), while the responsevariables were proximate composition andamino acids composition. The mean response values ofproximate composition and amino acids profile are presentedin Tables 3 and 4 respectively. The independent and responsevariables were fitted to the second-order model equation (Eq.4) and its goodness of fit examined using analysis of variance(ANOVA), coefficient of determination (R_2 and R_2adj), lack-offittest and analysis of residual. The predictive regressionmodels developed for the relationship between the dependent(y) and independent (X) in terms of proximate composition ofextruded snack is presented in Eq. 8 to13 for moisture, fat, fibre, protein, ash and carbohydrate respectively . The coefficients with single factor (X_1, X_2,and X_3) represent the independent effect of a particularvariable, while coefficients with two of the factors (X_1X_2,X_1X_3, and X_2X_3) and the ones with second-order terms ($X^2{}_1$,$X^2{}_2$, and $X^2{}_3$) represent interaction between the three factorsand quadratic effects respectively. A positive sign in front ofthe regression term is an indication of synergetic relationship,while negative sign indicates an antagonistic relationship.

$$\text{Protein} = 129.48 – 0.92X_1 - 3.31X_2 - 1.00X_3 + 0.01X_1^2 + 0.02X_2^2 + 0.002X_3^2 - 0.01X_1X_2 + 0.001X_1X_3 + 0.02X_2X_3 \ldots\ldots\ldots\ldots(8)$$

$$\text{Fat} = 34.38 - 0.10X_1 - 1.38X_2 - 0.24X_3 - 0.0002X_1^2 + 0.02X_2^2 + 0.001X_3^2 + 0.009X_1X_2 - 0.001X_1X_3 - 0.0001X_2X_3 \ldots\ldots(9)$$

Fibre=4.697-0.03X1-0.16X2-0.03X3+0.0004X1²+0.002X2²+ 0.000X3²+0.0001X1X2- 0.00X1X3+0.001X2X3…………(10)

$$Fibre=4.697-0.03X_1-0.16X_2-0.03X_3+0.0004X_1^2+0.002X_2^2+0.000X_3^2+0.0001X_1X_2-0.00X_1X_3+0.001X_2X_3 \quad (10)$$

$$Ash=0.291-0.05X_1+0.03X_2+0.05X_3+0.0004X_1^2+0.002X_2^2-0.00X_3^2+0.001X_1X_2-0.00X_1X_3-0.001X_2X_3 \quad (11)$$

$$Moisture= -5.08+0.099X_1+0.48X_2+0.09X_3+0.001X_1^2-0.01X_2^2-0.0003X_3^2+0.002X_1X_2-0.001X_1X_3+0.0003X_2X_3 \quad (12)$$

$$Carbohydrate=-64.02+0.998X_1+4.35X_2+1.14X_3-0.013X_1^2-0.04X_2^2-0.003X_3^2-0.01X_1X_2+0.001X_1X_3-0.02X_2X_3 \quad (13)$$

While the predictive models representing the relationshipbetween the extruder (process) variables and the responsevariables in terms of amino acids profile of the extruded snacks are outlined in the regression models equation 14 to23. The results of ANOVA performed on the models toevaluate the significance of the linear, quadratic andinteractive effects of the independent variables on thedependent variables are presented in Tables 5 and 6respectively for proximate and amino acid profilesrespectively. This analysis was done using the Fisher's F-test.

In this study, the ANOVA indicated significant ($p<0.05$)models since probability value is less than 0.05. Thecoefficients of determination (R_2 and R_2adj) results also showsthat the proximate composition predictive models R_2 valuesranged between 55.80 and 89.9% (Table 5) and R_2adj varyingbetween 44.5 and 87.3% . R_2 is the ratio of theexplained variation to the total variation and measures thedegree of fitness of a regression model (Filli *et al*. 2011); it therefore defines the proportion of thevariability in the observed response variables which isaccounted for by regression analysis (Filli *et al*., 2011). The closer the R_2 value is to unity, thebetter the empirical model fits the actual data (Zaibunnisa *et al.,* 2009), but the less the value of R_2,the less relevant the determinant variables in the model havein explaining variability observed in the response variables.Zaibunnisa *et al.* (2009) suggested that R_2 value should be atleast 80% to have good fit of a regression model. The resultsof this study therefore showed that the model for all theresponse variables were highly adequate to explain thevariability in response because they have satisfactory level ofR_2 which is higher than 80% and R_2adj that is close to R_2. Butthat of moisture content was less than 80% and is likely be asnon-significance of some terms added in the model.As repeated measurements were carried out during the datageneration, lack-of-fit test which indicate the significance ofthe replicate error in comparison with the model dependenterror was carried out. This test split the error sum of squaresinto two portions, one which is due to pure error and otherdue to lack-of-fit. F-test was then used to determine witherthe lack-of-fit test was significant or not. The resultsindicated non-significant ($p<0.05$) lack-of-fit in bothproximate and amino acid profiles (Tables 5 and 6). Nonsignificantlack-of-fit therefore is desired as a significant testindicates that there may be contributions in the regressionresponse relationship that are accounted for by the fittedmodels. It is appropriate therefore to conclude that the fittedmodels adequately approximates the responses and can beused satisfactorily for the prediction of any value of theresponses within the defined experimental range.

$$Lysine=6.95-0.04X_1-0.41X_2+0.06X_3+0.0001X_1^2+0.01X_2^2-0.0003X_3^2-0.001X_1X_2+0.001X_1X_3-0.0001X_2X_3 \quad 14$$

$$Histidine=-0.41+0.03X_1-0.20X_2+0.07X_3-0.0001X_1^2+0.01X_2^2-0.0001X_3^2+0.001X_1X_2-0.0004X_1X_3-0.00X_2X_3 \quad 15$$

$$Arginine=20.63-0.05X_1-0.44X_2-0.14X_3+0.0001X_1^2+0.01X_2^2+0.001X_3^2+0.002X_1X_2-0.0002X_1X_3+0.0001X_2X_3 \quad 16$$

$$Aspartic\ acid=12.61-0.14X_1-0.38X_2+0.04X_3+0.0001X_1^2+0.01X_2^2+0.00X_3^2+0.004X_1X_2+0.00X_1X_3-0.00X_2X_3 \quad 17$$

Serine=13.78-0.07X₁-0.29X₂-0.11X₃-0.00X₁²+

$$Serine=13.78-0.07X_1-0.29X_2-0.11X_3-0.00X_1^2+0.003X_2^2+0.0004X_3^2+0.002X_1X_2+0.0002X_1X_3+0.001X_2X_3 \quad ...18$$

$$Cystine=4.42-0.02X_1-0.09X_2-0.05X_3+0.00X_1^2+0.002X_2^2+0.0001X_3^2-0.0001X_1X_2+0.0004X_1X_3+0.001X_2X_3 \quad ...19$$

$$Valine=2.24-0.07X_1+0.20X_2+0.01X_3-0.00X_1^2-0.01X_2^2-0.00X_3^2+0.001X_1X_2+0.0004X_1X_3+0.001X_2X_3 \quad ...20$$

$$Methionine=1.96-0.03X_1-0.07X_2+0.01X_3+0.00X_1^2+0.001X_2^2-0.00X_3^2-0.00X_1X_2+0.0002X_1X_3+0.0003X_2X_3 \quad ...21$$

$$Iso\text{-}leucine=15.40+0.02X_1-0.31X_2-0.15X_3-0.00X_1^2+0.003X_2^2+0.00X_3^2-0.00X_1X_2+0.0002X_1X_3+0.0003X_2X_3 \quad ...22$$

$$Leucine=10.65-0.18X_1-0.36X_2+0.07X_3-0.0004X_1^2-0.0002X_2^2-0.001X_3^2+0.003X_1X_2+0.001X_1X_3+0.002X_2X_3 \quad ...23$$

3.3. Effects of Processing Variables on Proximate Composition

The results of mean observed values for proximatecomposition (moisture, protein, fat, fibre, ash and carbohydrate) for the extruded snacks is presented inTable 3. The moisture content ranged between 6.86 % and11.26%. The highest moisturecontent was recorded in sample corresponding to barreltemperature 120°C, moisture 25% and feed composition 60.59%and the least value in sample extruded at 100°C barreltemperature, 20% moisture and 15% feed composition content, themoisture content increase with increasing moisture content.It is also clear from these results that moisture content ofextruded sample depend mainly on the extrusion temperatureand on the amount of moisture of raw material and not onthe feed composition. These results suggested that theproduct had low moisture enough to have an extended shelflife. It has also been observed by several authors that in a dryfood systems with moisture content between 6% and 10%,there is a prolong shelf stability, and above this range, thestability of the system could be impeded by both chemicaland microbiological agents (Asare *et al.*, 2012). Protein content of foods are determined asnitrogen multiply by a factor (Nx6.25), and apparent proteincontent is not affected by extrusion temperature as nitrogen isnot affected by heat (Filli et al., 2011).The fat content ranged between 0.29 and 5.99%, with thehighest value observed in sample extruded at 140°C barreltemperature, 20% moisture and 50% feed composition. The increase infat content may be attributed to the possible raisingconcentration of some non-fat compounds formed by Millardreaction and/or caramelization which are insoluble in organicsolvent (El-Samahy et al., 2007). The fibre contents varied between 0.101 to 0.624%, with theleast value recorded in sample extruded at 120°C barreltemperature, 25% moisture and 32% feed composition and thehighest value observed in sample corresponding to 120°Cbarrel temperature, 25% moisture and 3.41% feed composition. During extrusion cooking process, positiveeffects of the process parameters on the total and solublefiber has been observed (Rashid, et al., 2015). Insolubledietary fiber decreased apparently as the process parameterschanges. These changes may probably be due to disruptionsof covalent and non-covalent bonds in the carbohydrate andprotein moieties leading to smaller and more solublemolecular fragments. The ash content which is the indicationof mineral content of the extruded snack varied between 2.05 and 3.27%, with the highest value observed insample corresponding to 140°C barrel temperature, 20%moisture and 15% feed composition and the leastvalue seen in sample extruded at 120°C barrel temperature,16.59% moisture and 32% feed composition. These results is contrary to anearlier observation by El-Samahy *et al.*, (2007), whoobserved significant increase in ash content when cactus pearconcentration was increased in a rice-cactus pear extrudedsamples. With respect to the carbohydrate valuesof the extruded snacks, the highest value was71.596% and the least value was 57.298%. Regression coefficients (β - this is the mean change in theresponse variables for a unit change in the independentvariables while holding the other predictors in the modelconstant) of the effects of the barrel temperature, feedmoisture content and feed blend composition on theproximate composition indicated negative linear andinteractive effects of these variables on the moisture content(Table 5) and the ANOVA indicates that both linear andquadratic effects were significant ($p<0.05$). This impliesthat increasing the feed composition may linearly increase themoisture content of the resulting extruded snack.The protein content coefficient of regression also showsthat there were significant ($p<0.05$) linear, quadratic andinteractive effects of the process variables on the proteinresults (Table 5). The negative linear effect suggests thatincreasing feed composition, barrel temperature andfeed moisture content resulted in decreased protein contentin the extruded snack, while the significance of the linear,quadratic and interactive terms indicated that these changesmay not be attributed to a single factor alone. Though, ithas been established that increasing protein basedcomponent of feed material directly increase productprotein content (Singh et al., 1991), butbecause of the effects of high temperature and low moisturecontent during extrusion, the protein content were reduceddue to denaturation. Though the protein content weresignificantly affected, at a steady state, the protein contentproduced in this study is above the minimum protein levelof 15.7% recommended by FAO/WHO/UNN (1985) forsupplementation feeding. The fat content were significantly($p<0.05$) affected by the linear, quadratic and interactiveterms, with the quadratic and linear in an antagonisticmanner (Table 5), while the ash content were negativelyaffected by the process variables. These results is contraryto an earlier findings by Obatolu (2002) and Filli et al.,(2011) who observed positive relationship between ashcontent and feed moisture content.

3.4. Effects of Processing Variables on Amino Acid Profile

The mean amino acid composition as affected by theprocessing parameters is presented in Table 4. The lysine value observed ranged between 3.13 and 4.89 g/100g protein for design points 14 and 11 representing 32% feed composition, 33.41% feed moisture content, 120^0C exit barrel temperature and 32% feed composition, 25% feed moisture content, 86.36^0C exit barrel temperature respectively. The observed values for lysine is slightly lower than the values (5.92 – 6.56g/100g protein) reported by Filli *et al.*(2011). The amino acid profile shows that lysine content of the extruded products increased as a result of the inclusion of the African yam bean flour. The iso-leucine value observed ranged between 2.35 and 3.52g/100g protein for design points 10 and 2 representing 60.59% feed composition, 25% feed moisture content, 120^0C exit barrel temperature and 50%feed composition, 20%feed moisture content, 100^0C exit barrel temperature respectively. The observed values for iso-leucine is higher than the value (0.25g/100g protein) reported by Samaila and Nwabueze (2013). For threonine, the observed values ranged between 2.90 - 4.09g/100g protein for design points 12 and 3 representing 32% feed composition, 25%feed moisture content, 153.64^0C exit barrel temperature and 15%feed composition,20%feed moisture content,140^0Cexit barrel temperature respectively. The observed values of phenylalanine ranged between 3.52-5.19g/100g protein for design points 10 and 1 representing 60.59%feed composition, 25%feed moisture content,120^0C exit barrel temperature and 15%feed composition, 20%feed moisture content,100exit barrel temperature respectively. For tyrosine, the observed values ranged between 2.32 – 3.48g/100g protein for design points 6 and 3 representing 50%feed composition, 30%feed moisture content,100^0Cexit barrel temperature and 15%feed composition,20%feed moisture content,140^{0}Cexit barrel temperature respectively. The observed values for leucine ranged between 6.40 – 8.48g/100g protein for design points 4 and 9 representing 50% feed composition, 20% feed moisture content,140^{0}Cexit barrel temperature and 3.41%feed composition, 25%feed moisture content,120^{0}Cexit barrel temperature respectively. For aspartic acid, the observed values ranged between 7.80 – 9.25g/100g protein for design points 2 and 4 representing 50%feed composition, 20%feed moisture content, 100^{0}C exit barrel temperature and 50% feed composition, 20% feed moisture content,140^{0}Cexit barrel temperature respectively. The observed values for arginine ranged between 5.02 – 6.38g/100g protein 10 and 1 representing 60.59%feed composition, 25%feed moisture content,120^{0}C exit barrel temperature and 15%feed composition, 20%feed moisture content,100exit barrel temperature respectively.

Table 3: Proximate composition of the extruded snacks samples (%)

Runs	Independent variables			Proximate composition (%)						
	X_1	X_2	X_3	Moisture	Protein	Fat	Fibre	Ash	CHO	Calorie (kcal/100g)
1	15	20	100.000	6.86	20.65	2.78	0.36	3.23	66.12	372.1
2	50	20	100.000	9.36	18.19	3.64	0.43	2.62	65.76	368.56
3	15	20	140.000	7.82	19.35	3.09	0.30	3.27	66.17	369.89
4	50	20	140.000	10.02	13.85	5.99	0.37	3.02	66.75	376.31
5	15	30	100.000	8.53	16.32	4.56	0.20	3.43	66.96	374.16
6	50	30	100.000	8.13	14.57	3.45	0.19	2.87	70.79	372.49
7	15	30	140.000	8.60	22.16	4.66	0.30	3.08	62.21	379.42
8	50	30	140.000	9.99	19.99	6.97	0.38	2.68	60.00	382.69
9	3.4069	25	120.000	8.69	30.04	0.29	0.62	3.06	57.30	351.97
10	60.591	25	120.000	11.26	16.29	3.99	0.14	2.74	65.58	363.39
11	32	25	86.364	8.81	14.50	4.66	0.24	2.61	69.18	376.66
12	32	25	153.636	8.43	15.54	3.06	0.13	2.71	70.13	370.22
13	32	16.59	120.000	9.82	13.86	3.98	0.15	2.05	70.14	371.82
14	32	33.40	120.000	8.39	17.50	3.03	0.13	2.92	68.02	369.35
15	32	25	120.000	8.95	13.47	3.09	0.10	2.79	71.60	368.09
Mean	NA	NA	NA	8.91	17.75	3.82	0.27	2.87	66.45	371.1413

X_1 = Feed composition (%), X_2 = Feed moisture content(%), X_3 = Barrel temperature (ºC), CHO = Carbohydrate, NA = Not applicable. Values are means of triplicate determinations. Experimental runs were randomized.

Table 4 :Amino acid profile for the extruded products (g/100g protein)

Runs	Amino acids composition (g/100 protein)																
	LYS	HIS	ARG	ASP	THL	SRN	GTA	PRL	GLC	ALN	CYS	VAL	MTN	ISL	LCN	TRS	PHA
1	4.15	2.32	6.38	8.87	3.38	3.20	12.29	3.25	4.12	3.81	1.11	4.02	1.21	3.20	7.54	3.31	5.19
2	3.82	2.19	5.19	7.80	3.10	2.79	10.38	3.02	4.02	3.56	0.90	3.32	0.80	3.52	5.93	2.48	3.78
3	5.03	2.22	5.70	9.03	4.09	3.03	13.64	3.36	3.92	4.10	1.18	3.89	1.31	3.00	6.90	3.48	4.93
4	3.79	2.51	5.28	9.25	3.61	3.29	12.54	3.13	4.51	3.60	0.83	3.62	0.78	2.71	6.40	2.98	5.02
5	3.66	3.02	6.21	8.90	4.01	2.94	10.73	2.90	3.66	3.60	0.97	3.53	0.99	2.48	6.95	3.31	5.37
6	3.60	2.51	5.02	8.30	3.41	2.82	11.30	3.02	3.51	4.31	0.83	3.35	0.86	3.10	6.72	2.32	3.96
7	4.10	2.73	5.79	7.80	3.29	3.00	10.87	2.78	4.02	3.73	1.04	3.65	1.15	2.94	7.34	2.98	-
8	3.90	2.32	4.94	8.55	3.98	3.61	13.22	3.25	3.89	4.02	0.97	4.02	0.99	3.03	8.01	3.31	3.61
9	4.21	2.19	5.79	7.90	3.18	3.00	11.23	3.25	4.20	3.94	0.97	4.20	1.20	2.90	8.48	2.81	4.58
10	3.38	2.51	5.02	7.86	3.21	2.61	11.01	2.90	3.61	4.01	0.83	3.50	0.80	2.35	5.90	3.14	3.52
11	4.89	2.60	6.04	8.46	3.32	3.06	12.01	2.32	3.61	4.31	0.83	3.50	0.91	2.90	8.10	3.31	3.96
12	3.82	3.02	5.62	9.03	2.90	3.29	10.09	3.02	4.02	3.52	1.18	3.83	1.15	3.03	7.01	3.14	3.87
13	3.52	2.19	6.30	7.83	3.41	3.20	11.09	2.90	3.51	4.10	1.04	4.02	0.78	3.29	6.66	2.81	4.31
14	3.13	2.32	5.62	8.02	3.21	3.50	10.59	3.25	4.02	3.60	0.97	3.77	0.94	3.07	7.13	2.65	3.78
15	3.82	2.41	5.19	8.11	3.29	2.91	10.52	2.55	3.76	3.94	0.90	4.02	0.99	2.81	7.40	3.14	3.79
Mean	3.92	2.47	5.60	8.38	3.43	3.08	11.43	2.99	3.89	3.88	0.97	3.75	0.99	2.96	7.10	3.01	3.98

LYS = Lysine, HIS =Histidine, ARG=Arginine, ASP=Aspartic acid, THL=Threonine, SRN=Serine, GTA=Glutamic acid, PRL=Proline, GLC=Glycine, ISL= Isoleucine, LEU =Leucine, VAL = Valine, MTN = Methionine, CYS = Cysteine, TRS = Tryosine, PHA = Phenylalanine.
X1 = Feed composition, X2 = Feed moisture content, X3 = Barrel temperature, BRT = Barrel temperature (ºC), FMC = Feed moisture content (%), FC = Feed composition (%).Values are means of triplicate determinations. Experimental runs were randomized.

Table 5 : Regression equation coefficients for the proximate composition of the extruded snacks

Term	Moisture Content (%)	Fat content (%)	Crude Protein (%)	Fibre (%)	Ash content (%)	Carbohy-drate (%)	Energy (Kcal/100g)
Constant	-3.55864	13.245	172.92 0	-0.3575	2.5712	-87.900	319.52
Linear							
X_1	0.01139*	-0.3329	-0.702	-0.0105*	0.0210	1.038	-0.676
X_2	0.24022	0.2850	-7.826	-0.0648*	-0.2346	7.9296	3.384
X_3	0.12921*	-0.1861	-0.762	0.0316	0.0494*	0.7236	0.419
Quadratic							
X_1^2	- 0.00084*	0.0019	0.002	-0.00014	0.0000	-0.0031	-0.011*
X_2^2	0.00657*	-0.0033	0.137*	0.00122*	0.00393	-0.1522	-0.125
X_3^2	-0.00013	0.0010*	0.002	-0.0002	-0.00012	-0.0025	-0.005*
Interaction							
X_{12}	0.00202	0.0089*	-0.004	0.0011*	0.00069*	-0.0077*	0.016
X_{13}	-0.00414	-0.0019	0.005*	-0.0001	-0.00016	0.0013*	0.019
X_{23}	0.00004	-0.0000	0.006*	0.0001	-0.00034	-0.0058*	0.008*
R^2	96.3	98.9	99.9	48.1	97.5	98.8	89.5
$R^2_{adj.}$	95.4	98.6	99.9	34.8	96.9	98.5	86.8
Lack-of-fit	0.83	0.43	1.850	0.03	0.17	1.31	2.65
Model	*	*	*	*	*	*	*

$^aY = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X^2{}_1 + \beta_{22} X^2{}_2 + \beta_{33} X^2{}_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$; X_1 =Feed Composition, X_2 = Feed Moisture Content, X_3 = Barrel Temperature, * = significant at 5% and 1% level of probability respectively,

3.5. Numerical Process Optimization

MINITAB's Response Optimizer was adopted forsimultaneous numerical optimization of the multipleresponses, to search for a combination of independentvariables levels that simultaneously satisfy the targetrequirement placed on each response and factors. Anuar *et al.*, (2013) and Gupta *et al* (2014) suggested that numericaloptimization require that goals (None, Maximum, Minimum,Target or Range) should be set for the independent variablesand response where all goals are combined into one desirablefunction. In this study, sets of conditions that will meet all thegoals, the independent variables (i) feed composition (15-50g/100), (ii) feed moisture content 20-30g/100) and (iii) barrel temperature (100-140°C) were all set withinrange, while protein, fibre and amino acid (lysine) were set at 3 and 5 respectively.Gupta *et al.* (2014) reported that the 'importance' score of agoal is within 1 to 5 and setting goal importance at 3indicates that the variable is considered to be equallyimportant, but Anuar *et al.* (2013) reported that when it is setat 5, the response target objective is to meet the objective ofgetting response at maximum level as applied in this study. The optimum independent variables were foundto be 120°C barrel temperature, 42.38g/100g feed composition and 22.8g/100g feed moisture composition whichproduce 26.65g/100g protein, 68.99 carbohydrate and lysine of 4.11mg/g protein. These results indicated that optimumvalues can be obtained in the extrusion of African yam bean and sorghum fortified with Moringa leaf powder that can satisfy both nutritional and functional requirementsof extruded snacks consumers.

4. CONCLUSION

In this study, different optimum extruded snacks wereproduced from several blends of African yam bean and sorghum. The African yam bean was added to improve its nutritionalquality. Response surface methodology and central compositedesign were adopted for the formulation and extrusioncooking. Statistically significant regression predictive modelswere fitted to demonstrate the relationship between the inputand output variables. Optimum levels of the input variablesthat favour optimum production of the extruded snacks with high protein and appreciable lysine level was achieved. Thisinformation can be adopted in the up-scale of extrusioncooking technology where African yam bean and sorghum are the mainingredients.

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