

Full Length Research Paper

Application of response surface methodology for the study of composition of extruded millet-cowpea mixtures for the manufacture of fura: A Nigerian food

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Accepted 22 November, 2011

A three-factor three level response surface methodology central composite rotatable design (CCRD) was adopted to study the effect of feed composition (X_1), feed moisture content (X_2) and screw speed (X_3) on the proximate, amino acid composition and sensory evaluation during extrusion of pearl millet and cowpea flour mixtures for the purpose of fura production. The mean observed value of protein for the fura-extrudates ranged from 11.23 to 16.76%. Analysis of variance indicates that linear and quadratic effects significantly ($P < 0.05$) affected the protein content of fura-extrudates. The mean value of lysine for the extrudates ranged from 5.11 to 6.56 g/100g protein and the methionine content ranged from 1.34 to 3.77 g/100 g protein. The regression models fitted to the experimental data showed high coefficients of determinants with $R^2 = 0.96, 0.94, 0.94, 0.85$ and 0.80 for protein (CHON), carbohydrate (CHO), fat (FAT), ash (ASH) and water (HOH) respectively. The R^2 values were 0.90, 0.85, 0.86, 0.92, 0.88, 0.85 and 0.93 for lysine, isoleucine, leucine, valine, methionine-cysteine, threonine and tryptophan, respectively. The coefficients showed good fit. The (CCRD) was effective in optimizing the process condition for fura as influenced by feed composition, feed moisture and screw speed. The importance of process variables on system parameters and physical properties could be ranked in the following order: Feed Composition (X_1) > Feed Moisture (X_2) > Screw Speed (X_3). The protein quantity and quality of fura was increased in terms of amino acid profile which justified the reason for fortification of millet with cowpea for fura production. The data obtained from the study can be used for the control of product characteristics and possible projection for the commercial production of fura or any enriched protein based food from the blends of pearl millet and cowpea.

Key words: Millet, cowpea, extrusion, fura, protein, amino acid, feed composition.

INTRODUCTION

Extrusion technology is one of the contemporary food processing technologies applied to foods (Harper and

Jansen, 1985) and can be applied to mitigate the problems associated with processing of traditional cereal based products in terms of improvement in functionality, physical state and shelf stability.

It offers many advantages over spray-drying and roller drying technologies in terms of preparation of ready-to-eat foods of desired shape, size, texture and sensory

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characteristics at very low processing cost (Guy, 2001; Sumathi et al., 2007). Extrusion is a powerful food processing operation, which utilizes high temperature and high shear force to produce a product with unique physical and chemical characteristics (Pansawat et al., 2008). This technology has some unique positive features when compared with other heat processes, because the material is subjected to intense mechanical shear (Singh et al., 2007). It is able to break the covalent bonds in biopolymers, and the intense structural disruption and mixing which facilitate the modification of functional properties of food ingredients and/or texturizing them (Asp and Bjorck, 1989; Carvalho and Mitchell, 2000; Singh et al., 2007). In addition, the extrusion process denatures undesirable enzymes; inactivates some anti-nutritional factors (trypsin inhibitors, haemagglutinins, tannins and phytates); sterilizes the finished product; and retains natural colours and flavours of foods (Fellows, 2000; Bhandari et al., 2001; Singh et al., 2007). The combination of high throughput rates, energy efficiency and versatility results in the potential for improved cost effectiveness and process rationalization over traditional production methods (Else et al., 1997). Excess water is not available in extrusion and the starch granules do not swell and rupture, as in classical gelatinization, but are instead mechanically disrupted by high shear forces and drastic pressure changes resulting in disappearance of native starch crystallinity, plasticization, expansion of the food structure, reduced paste viscosity, loss of water holding, increased reconstitutability of the extrudate, softer product texture and changes in colour (Harper, 1992; Kokini et al., 1992; Guha et al., 1998; Onwulata et al., 1998; Onyango et al., 2004). Generally, the functionality of extrudates is related to molecular modifications that occur during extrusion.

Pearl millet (*Pennisetum glaucum* [L.], R.Br.) is grown extensively in the dry areas of western and southern India and along the West African sub region including Nigeria where it is used as food for an estimated 400 million people (Hoseney et al., 1992). Pearl millet [*Pennisetum glaucum* (L) Leake] is an important cereal, contributing to the calorie and protein requirements of people in the semi-arid tropics (SAT). It is grown mostly in regions of low rainfall and is capable of withstanding adverse agro-climatic conditions. More than 80% of the production is used for human consumption, particularly in the SAT region of Africa and Asia. Several food preparations are made from pearl millet in Africa and India (Vogel and Graham 1979; Subramanian and Jambunathan, 1980a). In Nigeria, pearl millet has remained as a staple food in the form of gruels for the poor, especially in the northern part of the country. There have been limited efforts made by the scientific community to diversify its food uses by the application of

modern technology to upgrade of the traditional methods of contemporary food processing technology for millet utilization, despite the advancement in scientific research. The application of a contemporary technology for the traditional products in our fast growing social environment can enhance the development and acceptability of indigenous traditional based foods.

Grain legumes and cereal grains provide the major sources of calories and proteins for a large proportion of the world's population (NAS, 1975). Their amino acid profiles complement those of the cereal proteins, and this provides a good opportunity to restructure starch and protein based materials to manufacture a variety of textured convenience foods. While soybeans have had a comparative advantage over other legume seeds in terms of development, research and processing technology, however, little attention has been given towards other grain legumes like cowpea. There is a need to develop other sources of concentrated plant proteins (Mwasaru et al., 1999) which ideally should be crops that are widely grown in tropical countries. Dietary importance of legumes has been well established (Rocha-Guzman et al., 2006). The cowpea is probably the most popular grain legume in West Africa (Dolvo et al., 1975). In view of the increasing population growth in developing countries, the production and utilization of grain legumes will have to be increased. It is apparent that a variety of traditional foods can be prepared from grain legumes. Although, the chemical composition of cowpea has been reported in some publications (Jorg and Klein, 1989; Singh et al., 1993; Mwasaru et al., 1999), little information is available on the extrusion of millet-cowpea flour mixtures.

Fura is an example of the indigenous foods from millet in Nigeria. It is a traditional thick dough ball snack produced principally from millet or sorghum which is very common in Nigeria. The mode of preparation varies only slightly among different communities in the region, but the basic ingredient remains the same (i.e. millet or sorghum). Depending on the community, it is consumed with *nono* (local yoghurt produced from cow milk) or mashed in water before consumption in the form of porridge. But nowadays where scarcity of milk and milk product is pronounced, fura is consumed solely without the *nono*. In addition, the consumption and acceptability of fura has suffered some drawbacks because the method of processing has remained a home-based or artisanal activity that is carried out with rudimentary equipment and techniques, which is characterized by inconsistent product quality, poor hygiene, very short shelf life and unacceptable standards. Furthermore the product lacks process specifications governing composition and ingredients. Fura has a limited storage life with a range of 3-4 days at refrigeration storage (5°C), 1 to 2 days at room temperature (25°C) and 18 h at 35°C

(Jideani et al., 2002). Being a single cereal based product it is limiting in the essential amino acid lysine. Among all amino acids, lysine is the most limiting essential amino acid in cereal-based products, which are the majority of extruded products (Sin et al., 2006). As a means of resolving issues related with this limitation, due to their low protein content, fortification of millet with cowpea can go a long way in improving the protein quantity and quality of fura which is usually made from pearl millet solely. The inclusion of cowpea as a basic ingredient in producing fura through extrusion can improve its, nutritional quality, physical state and functionality. The poor quality of protein and high viscosity of traditional fura gruel makes it difficult to consume enough to meet both energy and protein requirements. Nkama and Filli (2006) and Filli and Nkama (2007), reported that extruded fura from cereal legume blends provided consumers with a fast, easy way to prepare nutritious fura which is similar to the traditional fura. Extrusion enhanced the water uptake of the product, with reduction in viscosity which is an indication of concomitant increase in nutrient density, but the process method was not optimized.

Modeling of extrusion processing involves consideration of process parameters, system parameters, and product properties. Thus, extrusion cooking modeling is a multiple input and multiple output process. Though mathematical modeling of food extrusion process has benefited from available information on plastic extrusion, modeling of quality changes during food extrusion is a difficult task. Response surface method (RSM) is a statistical mathematical tool which uses quantitative data in an experimental design to determine, and simultaneously solve multivariate equations, to optimize processes or products (Sefa-Dedeh et al., 2003); also it has been successfully used for developing, improving and optimizing processes (Wang et al., 2007).

The objectives of this work was to optimize process condition for fura by studying the effects of feed composition, feed moisture and screw speed on extrudate proximate composition and amino acid profile from pearl millet and cowpea flour mixtures using response surface methodology.

MATERIALS AND METHODS

Flour preparation from pearl millet

The process of flour preparation consisted of dry cleaning of millet i.e. winnowing using an aspirator Vegvari Ferenc (OB125, Hungary). The kernels were thereafter dehulled after mild wetting of the grain using a rice dehuller (India) at the Jimeta Main Market, Yola, Nigeria. After dehulling, the grains were washed and then dried in a Chirana convection oven model (HS 201A, Czech Republic) at 50°C for 24 h to 14% moisture content. The dried grain was milled

using a Brabender roller mill (OHG DUISBURG model 279002, Germany) equipped with a 150 µm screen.

Flour preparation from cowpea

The Cowpea seeds were steeped in tap water at 28°C for a period of 30 min to loosen the seed coat in a plastic bowl followed by decortication using the traditional pestle and mortar made of wood. The kernels mixed with the hulls were thereafter dried at 50°C to approximately 14% moisture content in a Chirana convection oven model (HS 201A, Czech Republic) for 24 h. The grain mass was winnowed to remove the hulls and remaining lighter materials using an aspirator Vegvari Ferenc (OB125, Hungary). The winnowed cowpea kernels were ground in a laboratory disc mill (made in Nigeria) to fine flour. The flour obtained was sieved using a 150 µm screen size Brabender (OHG Duisburg type, Germany) and the underflow was used for further research work.

Spice preparations

Kimba (Negro pepper) and ginger were sorted and cleaned manually before drying in a Chirana convection oven model (HS 201A, Czech Republic) at 60°C for five hours. The seeds were then pounded using the traditional pestle and mortar. The mass was ground and sieved using a 150 µm screen size.

Blend preparations and moisture adjustment

Millet flour (M_F) and cowpea flour (C_F) were mixed at various weight ratios, and the total moisture contents of the blends adjusted to the desired values with a mixer as described by Zasyplin and Tung-Ching Lee, (1998). Weights of the components to be mixed were calculated using the following formula:

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

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

$$W_X = M - C_{CF} - C_{MF} \quad (3)$$

C_{CF} and C_{MF} are the masses of cowpea flours (C_F) and millet flour (M_F), respectively, r_{CF} or r_{MF} are respective percentages of either cowpea flours (C_F) or millet flour (M_F) in the blend, d.b.; ($r_{CF} + r_{MF} = 100\%$); 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_{CF} and w_{MF} are the moisture contents of C_F and M_F , respectively. The blends were mixed in a plastic bowl with the addition of the spices Kimba and Ginger at 1% level, based on traditional formulation; and the whole packed in polyethylene bags which was kept in the refrigerator at 10°C overnight to allow moisture equilibration. The samples were however brought to room temperature before extrusion process.

Table 1. Independent Variables and Levels used for Central Composite Rotatable Design¹.

Variable	Symbol (X_i)	Coded variable level (x_i)				
		-1.68(∞)	-1	0	1	1.68(∞)
Feed composition (%)	X_1	3.2	10	20	30	36.8
Feed moisture (%)	X_2	16.6	20	25	30	33.4
Screw speed (Rpm)	X_3	116	150	200	250	284

¹Transformation of coded variable (x_i) levels to uncoded variables (X_i) levels could be obtained from $X_1 = 10x_1 + 20$; $X_2 = 5x_2 + 25$; $X_3 = 50x_3 + 200$.

Table 2. Experimental design extrusion experiment in their coded form and natural units^{1,2}.

Design point	Independent variables in coded form			Experimental variables in their natural units		
	(x_1)	(x_2)	(x_3)	(X_1)	(X_2)	(X_3)
1.	-1	-1	-1	10	20	150
2.	-1	+1	-1	10	30	150
3.	-1	-1	+1	10	20	250
4.	-1	+1	+1	10	30	250
5.	+1	-1	-1	30	20	150
6.	+1	+1	-1	30	30	150
7.	+1	-1	+1	30	20	250
8.	+1	+1	+1	30	30	250
9.	-1.68	0	0	3.2	25	200
10.	+1.68	0	0	36.8	25	200
11.	0	-1.68	0	20	16.6	200
12.	0	+1.68	0	20	33.4	200
13.	0	0	-1.68	20	25	116
14.	0	0	+1.68	20	25	284
15.	0	0	0	20	25	200

¹Duplicate tests at all design point except the centre point (0, 0, 0) which was carried out five times and the result averaged.

²Experiment was carried out in randomized order; (X_1) =feed composition (%), (X_2) = feed moisture (%) and (X_3) = Screw speed (rpm).

Experimental design and data analysis

A three-factor three levels central composite rotatable composite design [CCRD] (Box and Hunter, 1957) was adopted to study the effect of feed composition (X_1), feed moisture content (X_2) and screw speed (X_3) on the proximate composition and amino acid profile during extrusion of pearl millet and cowpea flour mixtures for fura production. The independent variables and their variation levels are shown in Table 1. The levels of each variables were established according to literature information and preliminary trials. The outline of the experimental layout with the coded and natural values are presented in Table 2. Homogeneous variances or homoscedasticity is a necessary pre-requisite for (linear) regression models. Therefore, a reduction in variability within the objective response (dependent variables) was by transforming the data to

standardized scores $z = \frac{x - \bar{x}}{s}$ where x = dependent variable of

interest; \bar{x} = mean of dependent variable of interest and s = standard deviation). For each standardized scores, analysis of variance (ANOVA) was conducted to determine significant differences among the treatment combinations. Also, data were analyzed using multiple regression procedures (SPSS, 2008). A quadratic polynomial regression model was assumed for predicting individual responses (Gacula and Singh, 1984; Wanasundara and Shahidi, 1996). The model proposed for each response of Y was:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (4)$$

where Y= the response X_1 = Feed Composition, X_2 = Feed Moisture, X_3 = Screw Speed, b_0 = intercepts, b_1, b_2, b_3 are linear, b_{11}, b_{22}, b_{33} are quadratic and b_{12}, b_{13} and b_{23} are interaction regression coefficient terms. Coefficients of determination (R^2) were computed. The adequacy of the models was tested by separating the residual sum of squares into pure error and lack of fit. For each

response, response surface plots were produced from the fitted quadratic equations, by holding the variable with the least effect on the response equal to a constant value, and changing the other two variables.

Extrusion exercise

Extrusion cooking was performed in a single screw extruder, model (Brabender Duisburg DCE-330, Germany) equipped with a variable speed D-C drive unit, and strain gauge type torque meter. The screw has a linearly tapered rod and 20 equidistantly positioned flights. The extruder was fed manually through a screw operated conical hopper at a speed of 30 rpm which ensures the flights of the screw filled and avoiding accumulation of the material in the hopper. This type of feeding provides the close to maximal flow rate for the selected process parameters (constant temperature, constant die and screw geometry but with three variable screw speeds) and three designed feed composition and feed moisture contents. A round channel die with separate infolding heater was used. The die used was a cone shaped channel with 45 degrees entrance angle, a 3 mm diameter opening and 90 mm length. The screw was a 3:1 compression ratio. The inner barrel is provided with a grooved surface to ensure zero slip at the wall. The barrel is divided into two independent electrically heated zones that is (feed end and central zone).

There is a third zone at the die barrel, electrically heated but not air cooled. The extruder barrel has a 20 mm diameter with length to diameter ratio (L:D) of 20:1. Desired barrel temperature was maintained by a circulating tap water controlled by inbuilt thermostat and a temperature control unit. The feed material was fed into a hopper mounted vertically above the end of the extruder which is equipped with a screw rotated at variable speed. The rotating hopper screw kept feed zone completely filled to achieve a 'choke fed' condition. Experimental samples were collected when steady state was achieved that is when the torque variation of ± 0.28 joules (Nm) or about (0.5%) of full scale (Likmani et al., 1991). The extrusion process consisted of 15 individual runs and was conducted randomly.

Moisture analysis

Moisture contents of raw and extrudates were determined as described by (AOAC, 1984). Triplicate determinations were carried out and the result averaged.

Crude fat determination

Crude fat of samples was determined using soxhlet fat extraction system (AOAC, 1984).

Crude protein determination

Protein content was determined by Kjeldahl Method (AOAC, 1984). Triplicate determinations were carried out and the result averaged.

Ash determination

Ash was determined by the method of AOAC (1984). Triplicate determinations were carried out and the result averaged.

Determination of crude fiber

Crude fiber was determined by the method of AOAC (1984). Triplicate determinations were carried out and the result averaged.

Determination of carbohydrates

The percentage carbohydrate was determined by difference (Egan et al., 1981).

Amino acid analysis

The method of Sotelo et al. (1994) was used in determining the amino acid content of the extrudates. One gram of sample was dissolved in 20 ml of 6N HCL. This was then poured into a hydrolysis tube with screw cap and hydrolyzed for 22 h under a nitrogen atmosphere. The acid was evaporated using a rotary evaporator and residue washed three times with distilled water. The extracted sample was dissolved in 1ml acetate buffer of pH 3.1. After dilution to a known volume, the hydrolysate was transferred into a Beckman system (model 6300) high performance amino acid analyzer. Amino acid scores were calculated as gram per 100 gram protein (g/100 g protein). Triplicate determinations were carried out and the result averaged.

Sensory evaluation

One hundred gram of pulverized extruded fura was added to 500 ml of water in a 1000 ml beaker each for all the 15 extruded fura samples. 100 g sugar was added to each of the preparations based on traditional recipe and these samples were used for sensory evaluation test, 20 untrained member judges (28 to 45 year old males and females) were employed. The prepared samples were placed on tables inside a plastic bowl provided with individual booths. The panelists were to taste and swallow each of the fura gruel and rinse their mouth with tap water between samples. The panelists were familiar with fura and they were asked to indicate their opinion on four sensory attributes, namely the colour, flavour, texture and the overall acceptability using Hedonic scale rating of 9 = liked extremely to 1 = disliked extremely. Acceptability scores using the mean of the observations were recorded.

RESULTS AND DISCUSSION

Model description

Studies were carried out using the response surface method in modeling the chemical composition and amino acid profile of extruded millet – cowpea blends as affected by the process variables feed composition level of cowpea added to millet (X_1), feed moisture (X_2), and screw speed (X_3) for the manufacture of fura. The mean values of proximate composition and amino acid profile as affected by the extrusion variables is presented in Tables 4 and 5, respectively. The independent and dependent variables were fitted to the second – order model equation and examined for the goodness of fit.

The analysis of variance were performed to evaluate the lack of fit and the significance of the linear, quadratic and interaction effects of the independent variables on the dependent variables (Table 6). The lack of fit test is a measure of the failure of a model to represent data in the experimental domain at which points were not included in the regression (Varnalis et al., 2004). Coefficient of determinant, R^2 , is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit (Haber and Runyon, 1977; Singh et al., 2007). It is also the proportion of the variability in the response variables, which is accounted for by the regression analysis (McLaren et al., 1977). When R^2 approaches unity, the better the empirical model fits the actual data. The smaller is R^2 , the less relevant the dependent variables in the model have in explaining the behavior of variation (Filmore et al., 1976; Myers and Montgomery, 2002). It is suggested that for good fit model, R^2 should be at least 80%. The results showed that the model for all the response variables were highly adequate because they have satisfactory levels of R^2 of more than 80% and that there is no significant lack of fit in all the response variables. If a model has a significant lack of fit, it is not a good indicator of the response and should not be used for prediction (Myers and Montgomery, 2002). We may probably conclude that the proposed models approximates the response surfaces and can be used suitably for prediction at any values of the parameters within experimental range.

Proximate composition

The mean result of proximate composition of fura extrudates is shown in Table 4. From the result it shows that the mean observed value of protein for the fura extrudates ranged from 11.23 to 16.76% suggesting proportional increase in protein content with fortification of pearl millet with cowpea flour. Protein content was measured as nitrogen ($N \times 6.25$); hence the apparent protein content was not affected by extrusion temperature, as nitrogen is not affected by heat treatment (Pelembé et al., 2002). The average value of the fat content for the fura extrudates showed a general low values which ranged from 1.54 to 3.56%. The mean observed values for the carbohydrates relatively remained within a narrow range of 74.12 to 77.34%. The carbohydrate level relatively remained high as expected as cowpea is equally high in carbohydrates. The ash content showed that it remained within a narrow range of 1.67 to 1.98%. Regression coefficients for objective responses for extruded fura composition are presented in Table 6. Analysis of variance (table not shown) indicates that linear and quadratic effects significantly ($P < 0.05$)

affected the protein content of fura extrudates as expected. The result indicated that increasing the amount of cowpea flour resulted in linear increase in the protein contents of extruded fura. The influence of feed moisture and screw speed independent variables linear terms indicated negative effects on the protein content but was not significant ($P < 0.05$). This negative effect suggests that increasing the feed moisture and screw speed resulted in decreased amount of protein content in the extrudate. This reduction in protein content may be attributed to the complex nature of interactions between extruder conditions, these changes might not be related to a single factor. Hence, the role of feed moisture, screw speed and other interactions of other parameters on the protein nutritional value is a point that obviously needs further investigation. The quadratic effect of feed composition showed significant ($P < 0.05$) influence on the protein content. The effect of square term on the protein content was a positive effect. However, there was no significant effect observed by the interaction terms on the protein content of extrudates. The regression analysis for the carbohydrate indicated that the linear effect of feed composition affected the carbohydrate contents significantly ($P < 0.05$). This result indicated that increase in the amount cowpea flour translates to lower values of carbohydrates of the fura extrudates. The linear effect of feed moisture and screw speed indicated insignificant ($P < 0.05$) effect on the carbohydrate content of extrudates. Experimental design points 5, 6, 7, 8 and 10 have attained the least protein contents if compared with the FAO/WHO/UNN (1985) minimum protein level of 15.7% recommended for supplementary mixtures of protein (Table 4).

The carbohydrate and fat content was significantly ($P < 0.05$) influenced by the linear term. This influence was negative effect suggesting that increase in the amount of cowpea flour resulted in the decrease in the carbohydrates and fat contents of fura extrudates. This effect is expected as millet flour is higher in the amounts of carbohydrates and fats than cowpea (Table 3). It appears that increasing the level of cowpea flour resulted in decreased fat content. Both millet and cowpea are low in fat content (Table 3). In addition, it may be attributed to the fact that cowpea flour is relatively lower in fat than millet flour (Table 3). An implication of this low fat content is the need for fat fortification in millet – cowpea extrudates, to meet up with the minimum fat requirement of 6% for complementary formulations (Mitzner et al., 1984). However, the low energy content as a result of low fat can be compensated for by the reduced viscosity due extrusion, therefore allowing a high level of solid matter in the gruel. The linear effect of feed composition indicated significant ($P < 0.05$) influence on the ash content. The effect was a positive, suggesting increase in the ash

Table 3. Proximate composition (%) of pearl millet and cowpea flours.

Analysis	Pearl millet flour (%)	Cowpea flour (%)
Protein	10.80±0.56	22.10±0.36
Carbohydrate	70.80±0.78	60.20±0.47
Fats	3.80±0.12	1.70±0.18
Ash	1.60±0.23	1.90±0.28
Crude fiber	1.80±0.15	2.10±0.21
Moisture	11.20±0.34	12.00±0.65

Table 4. Experimental design and observed values of proximate composition of the extruded millet-cowpea fura.

Independent variables ^b			Dependent variables ^a				
X ₁	X ₂	X ₃	CHON	FAT	CHO	ASH	HOH
10	20	150	12.67±0.34	3.23±0.34	76.89±0.44	1.76±0.32	5.43±0.34
10	30	150	12.88±0.54	3.22±0.25	77.12±0.98	1.76±0.12	5.23±0.21
10	20	250	12.87 ±0.23	3.21±0.65	77.23±0.78	1.67±0.14	5.17±0.32
10	30	250	12.65±0.46	3.24±0.34	76.67±0.67	1.82±0.23	5.45±0.43
30	20	150	15.77±0.48	1.67±0.21	75.63±0.65	1.92±0.21	5.01±0.28
30	30	150	15.75±0.76	1.66±0.43	75.67±0.87	1.91±0.24	5.05±0.35
30	20	250	15.67±0.35	1.69±0.42	75.34±0.43	1.96±0.22	5.67±0.65
30	30	250	15.76±0.46	1.54±0.41	75.32±0.56	1.98±0.45	5.34±0.24
3.2	25	200	11.23±0.25	3.56±0.33	77.34±0.43	1.67±0.16	5.67±0.43
36.8	25	200	16.76±0.54	2.02±0.28	74.12±0.87	1.98±0.18	5.23±0.26
20	16.6	200	14.54±0.66	2.88±0.25	75.34±0.59	1.76±0.31	5.23±0.46
20	33.4	200	14.34±0.28	2.87±0.65	75.65±0.78	1.78±0.34	5.56±0.32
20	25	116	14.34±0.76	3.01±0.35	75.67±0.65	1.88±0.21	5.26±0.65
20	25	284	14.36±0.87	2.67±0.28	76.45±0.67	1.78±0.14	5.32±0.21
20	25	200	14.32±0.51	2.87±0.25	75.78±0.58	1.89±0.25	5.54±0.22

^bX₁ = Feed composition (%); X₂ = Feed moisture (%) and X₃ = Screw speed (rpm). CHON = Protein; FAT = Fat; CHO = Carbohydrate; ASH = Ash; HOH = Water. ^aValues are means and ± standard deviation of triplicate determinations.

content as cowpea flour was added to millet flour. Though generally, the amount of ash was so low in the formulations of this study. Obatolu (2002) similarly observed low ash contents of mixtures of cereal/legume blends. This could be explained probably because cowpea flour relatively has more amount of ash content when compared with millet flour (Table 3). The regression models fitted to the experimental data showed high coefficients of determinants with R² = 0.96, 0.94, 0.94, 0.85 and 0.80 for protein (CHON), carbohydrate (CHO), fat (FAT), ash (ASH) and water (HOH), respectively. This indicated that the regression models could be considered adequate to study response tendencies of the extrusion variables for this study. Among the three extrusion variables, feed composition was analyzed to be most important factor affecting the proximate composition of fura extrudates.

carbohydrate (70.8%) and protein (10.8%) (Table 3). This study has shown increase in the protein content of millet as a result of fortification with cowpea flour. In addition legume proteins are rich sources of lysine and threonine (Alonso et al., 2000a; Carbonaro et al., 1997). From the view point of protein content, the basis of advocating cereal – based complementation as a method of improving the protein content of cereal – based traditional foods is justified by the results of this study.

Several workers have also reported marked improvements in the protein content of cereals when fortified with legumes (Akpapunam and Sefa – Dedeh, 1995; Sefa – Dedeh et al., 2003; Nkama and Filli, 2006; Sumathi et al., 2007; Curic et al., 2007; Hagenimana et al., 2007; Lazou et al., 2007; Kasprzak and Rzedzicki, 2008; Anton et al., 2009; Lazou and Krokida, 2010).

The major constituents of pearl millet flour are

Table 5. Observed amino acid profile of millet - cowpea furaextrudates (g/100 g protein).

Independent variables ^b			Dependent variables ^a							
X ₁	X ₂	X ₃	¹ Lysine	² I/leu	³ Leucine	⁴ Valine	⁵ Met+Cyt	⁶ Threonine	⁷ Tyr+Phe	⁸ Tryptophan
10	20	150	5.21±0.14	2.85±0.12	9.11±0.34	3.23±0.34	3.54±0.14	3.61±0.34	4.32±0.31	0.85 ±0.01
10	30	150	5.23±0.25	2.81±0.21	9.21±0.56	3.21±0.54	3.48±0.12	3.76 ±0.31	4.18±0.35	0.86 ±0.04
10	20	250	5.16±0.54	2.83±0.23	9.18 ±0.78	3.28±0.32	3.47±0.17	3.84±0.21	4.28±0.41	0.87±0.02
10	30	250	5.17±0.34	2.78±0.22	9.14±0.35	3.14±0.21	3.46±0.15	2.68±0.24	4.25±0.31	0.83±0.01
30	20	150	5.86±0.56	3.14±0.43	10.31±0.67	4.31±0.45	1.94±0.13	4.21±0.25	4.83±0.24	1.03±0.05
30	30	150	5.92±0.35	3.14±0.23	10.13±0.64	4.32±0.34	1.72 ±0.21	4.24±0.21	4.84±0.36	1.04±0.03
30	20	250	5.82±0.46	3.21±0.45	10.21±0.45	4.35±0.56	1.84±0.23	3.28±0.26	4.96±0.47	1.05±0.08
30	30	250	5.76±0.34	3.16±0.42	10.28±0.54	4.28±0.76	1.78 ±0.19	3.21±0.27	4.75±0.26	1.06±0.06
3.2	25	200	5.11±0.45	2.54±0.24	6.86±0.43	2.36±0.41	3.77±0.20	3.35±0.17	5.85±0.25	0.72±0.02
36.8	25	200	6.56±0.76	3.41±0.34	11.36±0.45	5.45±0.54	1.34±0.32	4.62±0.18	4.66±0.32	1.32±0.05
20	16.6	200	5.88±0.43	2.51±0.36	10.54±0.65	3.45±0.65	1.98±0.16	4.35±0.32	4.46±0.17	1.23±0.04
20	33.4	200	5.96±0.74	2.56±0.35	10.61±0.43	3.65±0.53	1.95±0.24	4.31±0.31	4.45±0.18	1.28±0.06
20	25	116	5.23±0.35	2.48±0.32	10.48±0.35	3.75±0.41	1.91±0.24	4.42±0.25	4.51±0.23	1.24±0.04
20	25	284	5.41±0.84	2.53±0.43	10.55±0.66	3.61±0.42	1.83±0.21	4.36 ±.19	4.46±0.29	1.27±0.02
20	25	200	5.36±0.65	2.54±0.21	10.51±0.57	3.46±0.35	1.84±0.17	4.32±0.42	4.44 ±0.31	1.23±0.07

^bX₁ = Feed composition (%); X₂ = Feed moisture (%) and X₃ = Screw speed (rpm). ^aValues are means and ± standard deviation of triplicate determinations. 1 = Lysine; 2 = Isoleucine; 3 = Leucine; 4 = Valine; 5 = Methionine + Cystine; 6 = Threonine; 7 = Tyrosine + Phenylalanine; 8 = Tryptophan.

Amino acid profile

The mean result of amino acid profile of the fura extrudates is shown in Table 5. As already known, lysine and methionine are generally limiting in cereal and legumes, respectively. The result showed that the mean observed value of lysine for the extrudates ranged from 5.11 to 6.56 g/100 g protein. The methionine content ranged from 1.34 to 3.77 g/100 g protein (Table 5). Regression coefficients for objective responses for fura extrudates amino acid profile are presented in

Table 6. Analysis of variance (table not shown) indicated that the amino acid content was influenced significantly (P < 0.05) by the linear and quadratic terms for lysine, leucine, valine and threonine. The linear effects of feed composition was positive for lysine content, which revealed that increase in the amount of cowpea resulted in increased amount of lysine content of extrudates. The lysine content was however, negatively influenced by the linear effects of feed moisture and screw speed. This result confirmed the same effects of feed moisture and screw speed on

protein content earlier mentioned. The quadratic effect of feed composition influenced the lysine content of extrudates significantly (P < 0.05). The result shows positive effect of quadratic term on the lysine contents of extrudates. The isoleucine content was influenced significantly (P < 0.05) by the quadratic term effect of feed composition. The result showed positive effect by the quadratic effect of feed composition on the isoleucine content. The leucine content of fura extrudates was influenced significantly (P < 0.05) by the linear term of feed composition and screw speed.

Table 6. Regression equation coefficients for objective responses^{a, b} proximate composition and amino acid.

Coefficient	¹ CHON	² CHO	³ FAT	⁴ ASH	⁵ HOH	⁶ Lys	⁷ I/leu	⁸ Leu	⁹ Val	¹⁰ Met-Cys	¹¹ Thr	¹² Try
Linear												
B ₀	0.6763*	-0.1687	-0.3058	0.4909	-0.1614	0.1439	-0.8439	0.5391	-0.4924	-0.9593	0.5579	-0.1520
B ₁	1.2885*	-0.1070*	-1.2543*	0.7721*	-0.5354	0.6652*	0.6502	-0.6906*	0.9077*	0.6728	0.8487*	1.0300*
B ₂	-0.1577	-1.6 52	-0.1326	0.0382	0.4713	-0.1228	0.1034	0.0797	0.0346	-0.0110	0.0036	0.0125
B ₃	-0.2644	-1.4042	0.3635	-0.0670	0.5893	-0.2204	0.0645	-0.4578*	0.0040	0.0272	0.0120	-0.0274
Quadratic												
B ₁₁	0.1079*	0.6207	0.1659	-0.3243	0.1789	0.4677*	0.7290*	-0.4450*	0.3363*	0.7450	-0.5623*	0.1635
B ₂₂	-0.2846	0.6896	-0.2142	-0.3731	-0.4877	0.0238	0.3054	-0.2390	0.3964*	0.2424	-0.0568	-0.0208
B ₃₃	-0.3460	0.0118	-0.2174	-0.0220	0.2889	0.2646	0.1417	0.0124	-0.1193	0.2077	-0.0759	0.04670
Interaction												
B ₁₂	-0.2489	0.5286	0.3432	0.1720	-0.7230	-0.1274	-0.2585	-0.2193	-0.0182	0.0204	-0.0208	0.0183
B ₁₃	-0.1344	-0.4699	-0.2243	0.5573*	0.2602	0.1982	-0.3244	0.1010	-0.0364	0.0530	0.0061	0.0037
B ₂₃	-0.1888	0.7271	-0.1729	-0.0894	-0.3155	0.3327	0.2658	0.3770	-0.0788	-0.0204	0.0134	-0.0366
R ²	0.9629	0.9392	0.9377	0.8487	0.8013	0.8970	0.8475	0.8550	0.9210	0.8790	0.8490	0.9290
Adjusted R ²	0.8815	0.8364	0.9381	0.6225	0.3425	0.8360	0.8943	0.880	0.8210	0.8740	0.8560	0.8380
Lack of fit	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Model	*	*	*	*	*	**	*	*	*	*	*	*

^a $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$, X₁ = Feed Composition, X₂ = Feed Moisture, X₃ = Screw Speed^b, ** Significant at P < 0.05 and P < 0.01, respectively; NS, not significant. 1=Protein2=Carbohydrate3=Fat4=Ash5=Water6=Lysine7=Isoleucine8= Leucine9=Valine10=Methionine-Cystine11=Threonine12=Tryptophan.

This effect was negative which suggested that there was decrease in the leucine content of extrudates as cowpea flour and screw speed was increased.

The R² = 0.90, 0.85, 0.86, 0.92, 0.88, 0.85 and 0.93 for lysine, isoleucine, leucine, valine, methionine – cystine, threonine and tryptophan respectively, suggesting good fit of the model (Table 6). From the result of this study, it was

concluded that the level of lysine content of fura extrudates increased generally as the amount of cowpea flour was increased. The content of the amino acids tyrosine, phenylalanine, isoleucine, valine and leucine increased as the level of cowpea flour was increased in the extrudates, from the regression analysis (Table 6). Similar observations have been observed by other authors for blends of cereals and legumes (Isaac

and Johnson, 1975; Obatolu, 1998; Obatolu, 2002). Nkama and Malleshi (1998) reported that lysine increased by 75% as a result of supplementation of millet with cowpea at (83:17) ratio. They also reported similar increase of other essential amino acids as a result of supplementation; these amino acids include histidine, threonine, valine and isoleucine. Legumes provide a larger protein intake and



Figure 1 (Plate 1 to 15): (1) 10% Cowpea, 20% moisture, 150rpm; (2) 10% Cowpea,30%moisture, 150rpm; (3) 10% Cowpea, 20% moisture, 250 rpm; (4) 10% Cowpea, 30% moisture, 250 rpm; (5) 30% Cowpea, 20% moisture, 150 rpm; (6) 30% Cowpea, 30%moisture, 150 rpm; (7)30% Cowpea, 20% moisture, 250rpm.;(8) 30% Cowpea, 30% moisture, 250 rpm; (9) 3.2% Cowpea, 25% moisture, 200 rpm; (10) 36.8% Cowpea, 25% moisture, 200 rpm; (11) 20% Cowpea, 16.6% moisture, 200 rpm; (12)20% Cowpea, 33.4% moisture, 200rpm; (13)20% Cowpea, 25% moisture, 116 rpm; (14) 20% Cowpea, 25%, moisture, 284 rpm; (15) 20% Cowpea, 25% moisture, 200 rpm.

amino acid balance when consumed with cereals, which significantly improves the protein quality (Bressani, 1975). Pelembe et al. (2002) reported that protein content of extrudates increased proportionally with the amount of cowpea flour in sorghum. The mean values of the amino acids profile from this study revealed that some of the essential amino acids were present in adequate amount if compared with the recommended values of FAO/WHO (1973).

Performance verification of model

The suitability of the model equation for predicting the

optimum response of dependent variables was tested for the optimal conditions. The optimum process conditions and their respective predicted value is presented in Table 8. The predicted values were found to be quite close to the respective experimental data.

Extrudate photographic responses

The visual effects of extrusion variables (feed composition, feed moisture and screw speed) can be seen as shown in Figure 1 (Plates 1 to 15). The effect of the independent variables on the expansion ratio and colour of extrudates was evident. The results shown in

Table 7. Sensory qualities of extruded millet – cowpea mixtures.

Independent variables ^b			Dependent variables ^a			
X ₁	X ₂	X ₃	Colour	Flavour	Texture	Overall/Acceptability
10	20	150	6.45 ^b	6.87 ^{ab}	6.67 ^b	6.50 ^b
10	30	150	6.30 ^b	6.70 ^b	6.43 ^b	5.34 ^c
10	20	250	6.54 ^b	6.88 ^a	6.88 ^{ab}	6.66 ^b
10	30	250	6.30 ^b	6.34 ^b	6.38	6.45 ^b
30	20	150	6.41 ^b	6.87 ^{ab}	6.56 ^b	6.55 ^b
30	30	150	5.50 ^c	6.12 ^b	6.34 ^b	5.59 ^c
30	20	250	5.40 ^c	6.34 ^b	6.41 ^b	5.65 ^c
30	30	250	5.60 ^c	5.55 ^c	5.65 ^c	5.78 ^c
3.2	25	200	6.35 ^b	6.78 ^b	6.57 ^b	6.44 ^b
36.8	25	200	5.36 ^c	5.56 ^c	5.65 ^c	5.34 ^c
20	16.6	200	7.30 ^a	7.56 ^a	7.54 ^a	7.55 ^a
20	33.4	200	5.89 ^b	5.76 ^c	5.79 ^c	5.68 ^c
20	25	116	6.89 ^{ab}	6.76 ^b	6.66 ^b	6.75 ^b
20	25	284	5.53 ^c	5.25 ^c	6.54 ^b	5.55 ^c
20	25	200	5.40 ^c	5.35 ^c	6.53 ^b	5.34 ^c

^bX₁ = Feed composition (%); X₂ = Feed moisture (%) and X₃ = Screw speed (rpm). ^aMean values in the same column with different letters are significantly different (P < 0.05).

Table 8. Summary of optimum levels of dependent and independent variables.

Dependable variable	^d Independent variable			Predicted value
	Feed composition (%)	Feed moisture (%)	Screw speed (rpm)	
¹ CHON	31.50	21.90	161.30	16.20 ^b
² CHO	22.30	30.90	194.80	77.33 ^b
³ FAT	25.60	32.70	248.25	5.56 ^b
⁴ ASH	22.50	29.20	199.90	3.65 ^b
⁵ HOH	24.80	30.20	242.10	5.64 ^b
⁶ LYSINE	10.15	33.17	187.91	5.97 ^c
⁷ ISOLEUCINE	26.10	28.90	188.40	4.43 ^c
⁸ LEUCINE	5.964	27.25	229.67	5.96 ^c
⁹ VALINE	6.619	24.74	211.90	5.08 ^c
¹⁰ MET-CYS	38.10	28.80	202.2	1.61 ^c
¹¹ THREONINE	29.10	25.20	182.20	2.53 ^c
¹² TRYTPHAN	43.41	31.18	259.95	1.52 ^c
¹³ TYR+PHY	32.30	28.34	220.23	4.78 ^c

^dX₁ = Feed composition (%); X₂ = Feed moisture (%) and X₃ = Screw speed (rpm). 1 = Protein; 2 = Carbohydrate; 3 = Fat; 4 = Ash; 5 = Water; 6 = Lysine; 7 = Isoleucine; 8 = Leucine; 9 = Valine; 10 = Methionine + Cystine; 11 = Threonine; 12 = Tryptophan; 13 = Tyrosine + Phenylalanine; b = (%); c = g/100 g protein.

the photographs described the changes occurred during extrusion as influenced by the extrusion variables.

Sensory evaluation

The statistical sensory qualities of millet – cowpea fura

extrudates is presented in Table 7. The results showed that design point 11 representing 20% feed composition (X₁), 16.6% feed moisture (X₂) and 200 rpm screw speed (X₃) recorded the highest value of acceptability for colour. This design point was significantly different (P < 0.05) from other extrudates with the exception of design point 13 representing 20% feed composition, 25% feed

moisture and 116 rpm screw speed. The flavour showed that there was no significant difference ($P < 0.05$) between design point 1, 3, 5, and 11 Table 7. The same design point 11 recorded the same pattern of highest acceptance for the flavor, texture and overall acceptability of extrudates. The design point 11 had the lowest processing extrusion feed moisture and recorded the highest sectional expansion and lowest bulk density (result not shown) probably influenced the acceptability of this sample more than others. Due to more expansion which resulted in fading of the dark colour of extrudate to brighter form might have made the sample much acceptable to the panelists. Colour changes in extruded products have been reported to be due to decomposition of pigments, product expansion causing colour fading and chemical reactions such as caramelisation of carbohydrates (Chen et al., 1991).

Conclusion

Designed experiments were conducted following RSM for the extrusion of pearl millet and cowpea blends using a single screw extruder. The decision to adopt extrusion cooking was motivated by the need to improve nutritional status, physical state and the functionality of the end product. RSM was effective for estimating the effect of three independent variables. The model equation developed can be used for predicting proximate composition and amino acid profile of millet – cowpea blends. From the result of this study, the level of lysine content of fura extrudates increased generally as the amount of cowpea flour was increased.

The content of the amino acids tyrosine, phenylalanine, isoleucine, valine and leucine increased as the level of cowpea flour was increased in the extrudates, from the regression analysis Extrusion resulted in dehydrated, precooked product that would require only reconstitution in either cold or warm water before consumption. Extrusion cooking of fura constituted a great improvement on the traditional product that is at high moisture content of between 60 to 75% and readily deteriorates on storage. Extrudates obtained in this study had moisture content less than 7 g/100 g and would not require refrigeration for storage. The predicted values were found to be quite close to the respective experimental data. The importance of process variables on system parameters and physical properties could be ranked in the following order: Feed Composition (X_1) > Feed Moisture (X_2) > Screw Speed (X_3). This technology permits the utilization and co-processing of foods from cereals and legume crop which are the major agricultural produce in Africa. The process variables suitable for processing of ready – to – eat extrudates from pearl millet

and cowpea were found to be feasible from the result of this study.

ACKNOWLEDGEMENT

The funding and support provided by INTERNATIONAL FOUNDATION FOR SCIENCE (IFS) is gratefully acknowledged and appreciated.

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