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Modeling of Residual Polyphenols, Phytic Acid and Protein Digestibility of Extruded Sorghum-Cowpea Formulated Foods

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Abstract
Blends of cowpea (10%, 20% and 30%) and sorghum flour were extruded at 20%, 22.5% and 25% moisture levels and 120°C, 140°C and 160°C barrel temperatures using a single-screw extruder. Response surface methodology with central composite face-centered (CCF) design was used to model the residual phytic acid and polyphenols, protein solubility and protein digestibility of the extrudates. Increasing the barrel temperature caused a reduction in protein solubility, residual polyphenols and phytic acid but increased the *in vitro* protein digestibility of the extrudates. The coefficients of determination (R^2) were 0.97, 0.99, 0.98 and 0.89 for polyphenols, phytic acid, protein solubility and protein digestibility respectively with non-significant lack of fit in all cases. The correlation coefficients (r^2) of observed and predicted values ranged from 0.99 to 0.98 suggesting a good fit for the model. Barrel temperature had the most effect on the responses. The second order polynomial was found appropriate for the prediction of polyphenols, phytic acid and protein digestibility of the sorghum- cowpea extrudates.
Keywords: response surface, digestibility, polyphenols, phytic acid, extrusion.

1. Introduction

Tannins have been reported to be one of the factors affecting the selection of sorghum for food processing (Nicholson, 1992 and Rooney, 1992). The polyphenols (tannins) in sorghum have been found to form complexes with proteins, reducing their biological value. Hamaker *et al*. (1987), Maclean *et al*. (1981) and Mertz *et al*. (1984), observed that even without tannins, the protein digestibility of cooked sorghum is lower than that of other major cereals. This decrease in the digestibility is thought to be caused by the formation of disulphide bonds during cooking, thereby creating less-digestible proteins.

It has been argued (Rooney, 1992) that all sorghums contain phenols which can affect the colour, appearance, and nutritional quality of the grains and their products. The phenolic compounds in sorghum can be divided into three groups: phenolic acids, flavonoids, and tannins. All sorghums contain phenolic acids and most contain flavonoids. According to Rooney (1992), only the brown-seeded, bird resistant sorghum contains condensed tannins. Rooney (1992) concluded that sorghums without pigmented testa do not have condensed tannins, and their nutritional value is 95% that of maize. The bird and fungi resistant nature of red sorghum makes it attractive to the Nigerian economy which is characterized by low technology of food processing and preservation.

Tannins and phytates have been found to reduce both protein and mineral bioavailability respectively in the diet (Reddy *et al*., 1989 and Salunkhe *et al*., 1990). Condensed tannins are located mainly in the outer layers (bran) of cereal grains and seed coats or testa of legumes and other seeds of higher plants. Tannins are reported to interact with proteins (both enzyme and non- enzyme proteins) to form complexes, resulting in inactivation of digestive enzymes and protein insolubility (Reddy *et al*., 1985, and Salunkhe *et al*., 1990). The result is lowered feed efficiency, growth depression and decreased iron absorption. Other deleterious effects of excess consumption of tannins include damage to mucosal lining of the gastrointestinal tract, alteration in the excretion of certain cations and increased excretion of proteins and essential amino acids (Reddy and Pierson,1994). Phytate works in a broad pH-region as a highly negatively charged ion and therefore its presence in the diet has a negative impact on the bioavailability of divalent and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} (Wu *et al*., 2009).

Extrusion cooking of sorghum is reported to have improved digestibility of sorghum (Fapojuwo *et al*., 1987). Alteration of pH before extrusion further improved digestibility. Gomez *et al.* (1988) extruded three sorghum varieties containing different amounts of amylose. According to the workers, different amylose/amylopectin ratios in the sorghum meal did not affect the *in vitro* protein digestibility of the extrudates; however, digestibility of extrudates increased as extrusion moisture content decreased. Extrusion technology with its numerous advantages over traditional and conventional methods (Rizvi *et al*., 1995) is one of the most versatile and energy efficient processes currently contributing solutions to world hunger and nutritional problems (Hauck, 1981).

Most Nigerian households spend more than 75 % of their income on food alone, indicating a high prevalence of food insecurity (NPC, 2001). Many Nigerians do not have access to animal protein because of its high cost. The utilization of extrusion cooking and supplementation of sorghum flour with plant protein from cowpea flour in the production of a breakfast cereal is likely to increase protein consumption of the population.

The major objective of this work was to produce an instant (extruded) breakfast cereal from sorghum-cowpea blends with low residual phytic acid and polyphenol content and improved protein digestibility.

2. Materials and Methods

2.1 Procurement of raw materials

The red sorghum variety (Chakalari red), was obtained from Maiduguri Monday market. Cowpea (var Kananede) was obtained from the Mubi main market.

2.2 Preparation of sorghum flour

About 15 kg of sorghum grains were cleaned using a laboratory aspirator (Vegvari Ferenc Type OB125, Hungary) to remove stalks, chaff, leaves and other foreign matter. They were then washed with treated tap water in plastic basins and sun dried on mats for 2 days (at 38 ^{o}C and relative humidity of 27.58 %) to 12 % moisture. This was then dehulled using a commercial rice dehuller (Konching 1115, China) and milled using an attrition mill (Imex GX 160, Japan). The flour was sieved to pass mesh number 25 (BS, 1985) before packing in polythene bags for further use.

2.3 Preparation of cowpea flour

About 3 kg of cowpea was soaked in water for 10 min to loosen the seed coat. The kernels were then cracked in a mortar with pestle. The seed coat was then washed off in excess water. The beans were oven dried (Model: Chirana HS 201A, Hungary) at 80 ^{o}C to 12% moisture content and milled into flour (Imex GX 160, Japan) which was sieved to pass mesh number 25 (BS, 1985) before packing in polythene bags for further use (Filli *et al*., 2010).

2.4 Blending of sorghum flour with cowpea flour and moisture adjustment

Sorghum flour was blended with cowpea flour in varying proportions (10%, 20%, 30% cowpea). The individual moisture contents of the cowpea and sorghum flours were determined (on dry weight basis) using the hot air oven method (Egan *et al*., 1981) and then the total moisture of the blends adjusted to the desired level according to Zasypkin and Tung-Ching (1998), using the formula below. The blends were mixed using a laboratory mixer (Hobert, Model: A200) and the moisture allowed to equilibrate for one hour before extrusion.

$$C_{cf} = [r_{cf} \times M \times (100-w)]/ [100 \times (100-W_{cf})]$$

$$C_{sf} = [r_{sf} \times M \times (100-w)]/ [100 \times (100-W_{sf})]$$

$$W_x = M- C_{cf} – C_{sf}$$

Where C_{cf} is the mass of cowpea flour (g); $C_{sf,}$ the mass of sorghum flour (g); $S_{f,}$ and C_f are sorghum flour and cowpea flour respectively; r_{cf} and r_{sf} are the cowpea flour (%) and sorghum flour (%) respectively; M, the total mass of the blend (g); w is the moisture content of final blend (%); W_x is weight of water added (g); $W_{cf\ ,}$ the moisture content of cowpea flour (%); while W_{sf} is the moisture content of sorghum flour (%).

2.5 The extrusion process

Extrusion cooking was done in a single screw extruder (Model: Brabender Duisburg DCE-330), equipped with a variable speed DC drive unit and strain gauge type torque meter. The extruder was fed manually through a screw operated conical hopper. The hopper which is mounted vertically above the end of the extruder is equipped with a screw which was adjusted to 139 rpm. The samples were extruded at a screw speed of 200 rpm; 2.0 mm die diameter, 2 bars pressure and a length/diameter ratio of 20:1. Experimental samples were collected when steady state (constant temperature and torque) was achieved. Variables considered were feed composition, feed moisture content and temperature of extrusion. Extrudates were kept on stainless steel work benches overnight to dry. They were then packaged in polythene bags prior to analysis.

2.6 Experimental design

The Central Composite Face-Centred Design (CCFC) used in this work was produced using MINITAB 14 statistical software (MINITAB 14, 2003). Table 1 shows the process variables and their levels used in the design. The experimental matrix used in the study, based on central composite face-centred design, is as shown in Table 2. The experimental space had fourteen star points and six central points, making a total of twenty runs. The data obtained from the study was fitted to the second-order polynomial regression model (Annor *et al*., 2009) of the form:

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

Where X_1, X_2 and X_3 are feed composition (cowpea flour), feed moisture and barrel temperature, respectively; b_o is the regression constant; b_1, b_2 and b_3 are linear regression terms; b_{11}, b_{22} and b_{33} are quadratic regression terms; b_{12}, b_{13} and b_{23} are the cross-product regression terms; ε is the error term. MINITAB Version 14 was used in the design of the experiment as presented in Table 2.

Table 1: Independent variables and their levels of replication

Parameters	code	Levels of replication		
		-1	0	+1
Cowpea flour (%)	X_1	10	20	30
Feed moisture (%)	X_2	20	22.5	25
Temperature (°C)	X_3	120	140	160

Table 2: Central composite face centered (CCF) design matrix and the independent variables in their natural forms.

Runs	X_1	X_2	X_3	Feed composition (%)	Feed moisture (%)	Extrusion temp.(°C)
1.	-1	-1	-1	10	20	120
2.	+1	-1	-1	30	20	120
3.	-1	+1	-1	10	25	120
4.	+	+1	-1	30	25	120
5.	-1	-1	+1	10	20	160
6.	+1	-1	+1	30	20	160
7.	-1	+1	+1	10	25	160
8.	+1	+1	+1	30	25	160
9.	-1	0	0	10	22.5	140
10.	+1	0	0	30	22.5	140
11.	0	-1	0	20	20	140
12.	0	+1	0	20	25	140
13.	0	0	-1	20	22.5	120
14.	0	0	+1	20	22.5	160
15.	0	0	0	20	22.5	140
16.	0	0	0	20	22.5	140
17.	0	0	0	20	22.5	140
18.	0	0	0	20	22.5	140
19.	0	0	0	20	22.5	140
20.	0	0	0	20	22.5	140

2.7 Protein solubility

The Malaysian Standard method (1997) reported in Annor *et al.* (2009) was used to determine the protein solubility of samples. One and a half grams (1.5 g) of the sample was weighed into a beaker and 75 ml of 0.2% (0.36 N, pH 12.5) potassium hydroxide was added. The sample was then stirred for 20 min on a magnetic stirrer plate and centrifuged (Model: Hettich Zentrifugen D-7200 Type 2008) at 2,700 rpm for 15 min. The supernatant was then filtered through glass wool into a beaker, being careful to avoid transferring residue. It was centrifuged again and 15ml supernatant was transferred into two Kjeldahl tubes for duplicate analysis (this gives 0.3g aliquot of the original sample); 12.5 ml concentrated sulphuric acid and 2 ml hydrogen peroxide was added to each tube for nitrogen determination by the Kjeldahl method. The total nitrogen of the original sample was also determined. Protein solubility was expressed as the soluble protein fraction (from supernatant) as a percentage of the total protein in the sample.

$$Protein\ solubility = \frac{protein\ in\ filtrate}{total\ protein\ in\ sample}\frac{100}{1}$$

2.8 Protein digestibility

This was determined according to the procedures of Onyango *et al*. (2004b). In this procedure, 200 mg sample was transferred to a 100 ml Erlenmeyer flask containing 35 ml 0.1M sodium citrate tribasic dehydrate (pH 2.0) with pepsin (1.5 g pepsin/litre). The mixture was incubated for 2 hrs in a water bath at 37 °C, shaken every 20 min and then centrifuged (Model: Hettich Zentrifugen D-7200 Type 2008) at 6,000 g for 15 min. The residue was collected on a nitrogen free filter paper and washed with 10 ml phosphate buffer (pH 7.0). The filter papers were dried at 108 °C for 3 hrs. The dried residue was analyzed for nitrogen using Kjeldahl method earlier discussed.

$$\%\ \textit{in vitro}\ \text{protein digestibility} = \frac{CP1 - CP2}{CP1}$$

Where:

CP1 = Total protein of extrudate

CP2 = Total protein after digestion with pepsin

2.9 Phytic acid

Phytate (phytic acid) content of samples was determined by the method described by Davies and Reid (1979). One gram of each sample (which was finely ground) was extracted in 40 ml of 0.5 M nitric acid for one hour. These were filtered and 5.0 ml of standard ferric chloride solution (2.0 mg/l) was added to each filtrate and incubated at 100 ^{o}C for 20 min. This was again filtered and 3 ml 0.004 M ammonium thiocyanate added to the filtrate. The absorbances of the standard ferric chloride solution and the free Fe^{3+} remaining in solution were read on a spectrophotometer (Jenway Model 6300) at 600 nm. The results were converted to milligrams of phytate using the 4.0 to 6.0 atomic ratio for iron to phosphorus (Fe: P) in ferric phytate (Garcia - Estepa *et al*., 1999).

2.10 Polyphenol determination

The Prussian blue assay for the determination of total polyphenolics (Price and Butler, 1977) was used. Ground samples (0.035g milled to pass through 1.0 mm sieve mesh) were extracted with 5 ml absolute methanol for 30 min. This was centrifuged (Model: Hettich Zentrifugen D-7200 Type 2008) at 6000 g for 15 min the supernatant was diluted 100 times with distilled water and then mixed with 3 ml 0.1 M $FeCl_3$ in 0.1 N HCl for 3 min followed by timed addition of 3 ml 0.008M potassium ferricyanide. The absorption was read on a spectrophotometer (Jenway Model 6300) at 720 nm.

Standard solutions prepared according to Gomez *et al.* (1997) were used to draw a standard curve. To prepare the solutions, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and1.0 ml of the stock catechin hydrate (Sigma Chem., Co.) solution containing 1.0 mg/l were pipetted into 10 test tubes respectively and made up to 1.0 ml with methanol, except the test tube containing 1.0 ml catechin solution. This was equivalent to 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg/l. This was further converted to milligrams of catechin hydrate per gram sample or per hundred-gram sample. The standard curve was used to estimate the concentration of the polyphenols in the samples.

2.11 Statistical analysis

MINITAB version 14 statistical analysis software (MINITAB 14, 2003) was used in the statistical analysis of data. Multiple regression analysis was used to analyse the various responses. A test of lack of fit, coefficient of determination (R^2) and correlation coefficient were used to determine the adequacy of the regression models. Analysis of variance (ANOVA) was used to establish statistical significance of the model. Correlation analysis was used to test the relationship between the predicted and observed values. Numerical optimization and interactive graphs were used to optimize the various input variables and responses.

3. Results and discussion

3.1 Protein solubility

The protein solubility of the extrudates ranged from 12.9 to 19.05% (table not shown). Maximum protein solubility was obtained from Run 4 (30:25:120). The lowest solubility was from Run 1(10: 20: 120). The increase in protein solubility of the extrudates as seen in the results could be explained by increase in cowpea flour in the feed. Pelembe *et al*. (2002) similarly observed that nitrogen solubility index increased as amount of cowpea was increased in the feed. This was explained by the higher protein content of cowpea and the fact that cowpea proteins are more water soluble than those of sorghum (Chavan *et al*., 1989). The negative effect of barrel temperature increase on protein solubility may be due to coagulation which usually occurs at higher temperatures rendering proteins insoluble. The digestibility and bioavailability of proteins are functions of its solubility (Annor *et al*., 2009). Iwe *et al*. (2001) stated that protein solubility is an important target parameter in the animal feed industry where it is used to characterize the protein quality of raw materials. Regression coefficients for protein solubility of sorghum-cowpea extrudates are presented in Table 3. Protein solubility of the extrudates was significantly affected by the linear and quadratic effects of barrel temperature with an R^2 value of 0.98 and a non-significant lack-of-fit. Joglekar and May (1987) recommended an R^2 value of 0.80 for a good fit. The coefficient of determination (R^2) value of 0.80 was adopted in this work. The observed and predicted values were also in close agreement ($r^2 = 0.99$) suggesting a good fit for the model. The model was therefore found adequate in describing the protein solubility of the extrudates. It could therefore be used as a predictor for the process. The linear effect of barrel temperature had the most influence on the protein solubility of the extrudates. The optimum water solubility of 14.58% was found at 10% feed composition, 20% feed moisture and 120 ^{o}C barrel temperature. The regression equations for the various responses are presented below:

Protein solubility = $126.9321 - 0.1885X_1 - 1.872X_2 - 1.2617X_3 + 0.0056X_1^2 + 0.0308X_2^2 + 0.004X_3^2 + 0.0083X_1X_2 + 0.003X_2X_3$ ($R^2 = 0.98$).

Protein digestibility = $56.7564 + 0.0007X_1 + 0.0843X_2 + 0.1258X_3 + 0.009X_1^2 + 0.0018X_2^2 - 0.0007X^2_3 - 0.0169X_1X_2 + 0.0017X_1X_3 + 0.0028X_2X_3$ ($R^2 = 0.89$).

Polyphenols = $31.1899 - 1.0231X_1 + 11.0021X_2 + 0.1569X_3 + 0.0251X_1^2 - 0.0246X_2^2 + 0.003X_3^2 + 0.0094X_1X_2 - 0.0019X_1X_3 - 0.0679X_2X_3$ ($R^2 = 0.97$).

Phytic acid = $371.741 - 0.5649X_1 + 4.0429X_2 - 4.1845X_3 + 0.0076X_1^2 - 0.0937X_2^2 + 0.0116X_3^2 - 0.0118X_1X_2 + 0.0038X_1X_3 + 0.0041X_2X_3$ ($R^2 = 0.99$).

Table 3: Regression coefficients for tannins, phytic acid and protein digestibility of sorghum-cowpea extrudates.

Coefficient	Tannins	Phytic acid	Protein solubility	Protein digestibility
Linear				
b_o	-31.899	371.741	126.932	56.756
b_1	-1.023	-0.565	-0.1885	0.0007
b_2	11.001	4.043	-1.872	0.0843
b_3	0.1569	-4.185	-1.2617*	0.1258
Quadratic				
b_{11}	0.0251	0.008	0.0056	0.009
b_{22}	-0.0246	-0.094	0.0308	0.0018
b_{33}	0.0030	0.012	0.004*	-0.0007
Interaction				
b_{12}	0.0094	-0.0118	0.0083	-0.0169
b_{13}	-0.0019	0.0038*	___	0.0017
b_{23}	-0.0679**	0.0041	0.003	0.0028
R^2	0.9694	0.9967	0.9775	0.8901
Adjusted R^2	0.9419	0.9938	0.9573	0.7913
Lack of fit	NS	NS	NS	NS

$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}(X_1)^2 + b_{22}(X_2)^2 + b_{33}(X_3)^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + \varepsilon$; X_1 = Feed composition, X_2 = Feed moisture, X_3 = Extrusion temperature; * Significant at $p \leq 0.05$, and **$p \leq 0.01$ respectively, NS = not significant.

The response surface curves for protein solubility of extrudates in shown in Figure 1. Protein solubility of the extrudates increased sharply as the feed composition increased but decreased as the barrel temperature was increased. It however slightly decreased as the barrel temperature was increased.

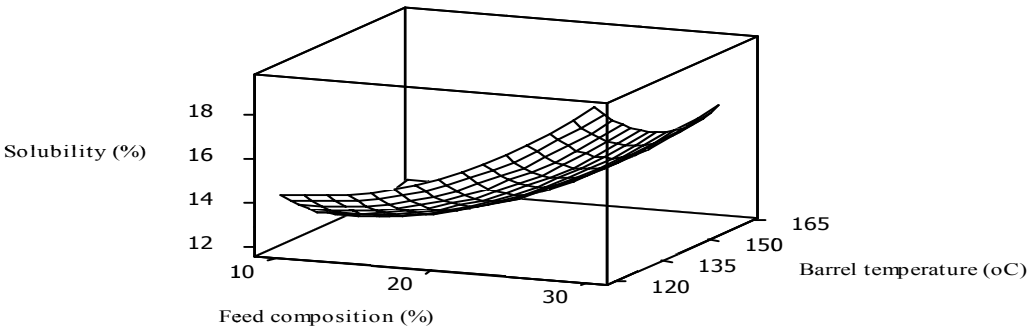

Figure 1: Response surface plot of effect of feed composition and barrel temperature on the protein solubility of extrudates.

3.2 Protein digestibility

Protein digestibility of the extrudates varied from 75.1 to 84.16% (table not shown). Maximum protein digestibility was obtained from Run 10 (30:22.5:140) while the least digestibility was from Run 9 (10:22.5:140). Feed composition when combined with feed moisture caused a progressive increase in the digestibility of the extrudates. Barrel temperature had the most effect on protein digestibility of the sorghum-cowpea extrudates. From the results it could be seen that protein digestibility of sorghum-cowpea extrudates generally improved (from 51%) as the sorghum cowpea blends were extruded. Hamaker and Bugusu (2003) suggested the disruption of the protein bodies in sorghum by the application of shear forces to improve digestibility and functionality of sorghum protein. Extrusion cooking of sorghum was earlier reported to improve protein digestibility of sorghum (Fapojuwo *et al*., 1987). Temperature was the key extrusion variable that influenced digestibility. Gomez *et al.* (1988) similarly reported improved protein digestibility when sorghum was extruded. Extrusion cooking improves *in vitro* protein digestibility by promoting thermally induced cross-links among sub-units of proteins, which exposes the enzyme-access sites of the protein. It inactivates antinutritional factors such as trypsin inhibitors, phytates and polyphenols that impair digestion. Even sorghum, whose *in vitro* protein digestibility declines on wet cooking, shows improved digestibility after extrusion cooking (Fapojuwo *et al*., 1987; Gomez *et al*., 1988 and Onyango *et al*., 2004b). Regression coefficients for protein digestibility of sorghum-cowpea extrudates are shown in Table 3. Protein digestibility of the extrudates was not significantly influenced by any of independent variables. The R^2 value for

protein digestibility was 0.89 with a non-significant lack-of-fit. The observed and predicted values were also in close agreement with r^2 value of 0.98 suggesting a good fit for the model. Extrusion temperature and feed composition had significant ($p \leq 0.05$) effect on protein digestibility of extrudates. The optimum protein digestibility of 72% was obtained at 20.62% feed composition, 20.6% feed moisture and 127.5 ^oC barrel temperature. The response surface plots for protein digestibility of extrudates in shown in Figures 2 (a) and (b). Protein digestibility increased to a maximum of 78% as the feed moisture increased to 22.55% and started to decline as the feed moisture was further increased. It similarly increased with increase in barrel temperature up to a peak at 78% and barrel temperature of 135^oC but started to decline as the barrel temperature continued to increase.

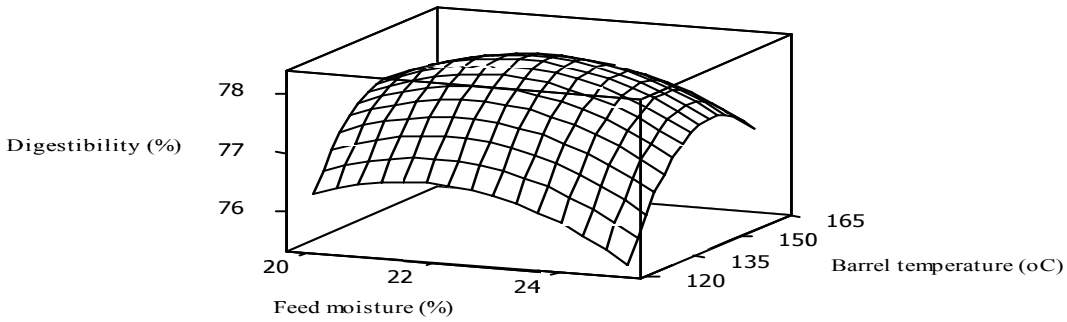

Figure 2(a): Response surface plot of effect of feed moisture and barrel temperature on the protein digestibility of extrudates.

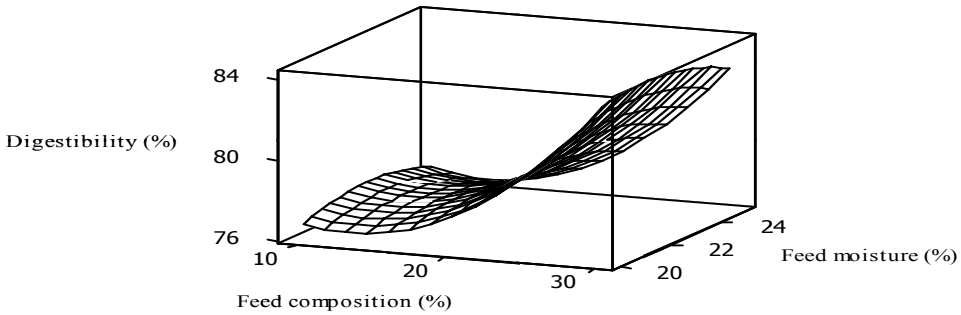

Figure 2(b): Response surface plot of effect of feed composition and moisture on the protein digestibility of extrudates.

3.3 Polyphenols (tannins)

The residual tannin content of the extrudates varied from 49.64 to 79.76 mg/100g (table not shown). Maximum polyphenol content was obtained from Run 4 (30:25:120) while the least residual polyphenol content was from Run 8 (30:25:160). Residual tannin content sharply decreased to a minimum (50 mg/100g) at 25% cowpea addition and tended to increase as cowpea flour was increased in the feed. The results show that extrusion cooking generally reduced the polyphenol levels in the raw sorghum-cowpea blends (442.1 to 485.3 mg/100g). Tannins form insoluble complexes with divalent ions in the gastrointestinal tract, lowering their bioavailability. Shinde *et al.* (1991) and Nkama and Gbenyi (2002) reported that during roasting, total phenols and tannins decrease. This corroborates the effect of high temperature on tannins reported in this work. Regression coefficients for polyphenols of sorghum-cowpea extrudates are presented in Table 3. The residual polyphenol content of the extrudates was significantly ($p<0.01$) affected by the negative interaction effects of feed moisture and barrel temperature. The R^2 value was 0.97 with a non-significant ($p>0.05$) lack-of-fit. The R^2 value for polyphenols of the sorghum-cowpea extrudates was high (>0.8). The observed and predicted values were also in close agreement with r^2 of 0.98 suggesting a good fit. The model was therefore found adequate in describing the residual polyphenols of the extrudates. The optimum polyphenol content of 62 mg/100g was found at 10% feed composition, 21% feed moisture and a barrel temperature of 138.5 ^oC. The response surface plots of residual tannin (polyphenol) content of extrudates in shown in Figure 3. The interaction effects of feed moisture and barrel temperature showed the most influence on the residual polyphenol content of the extrudates.

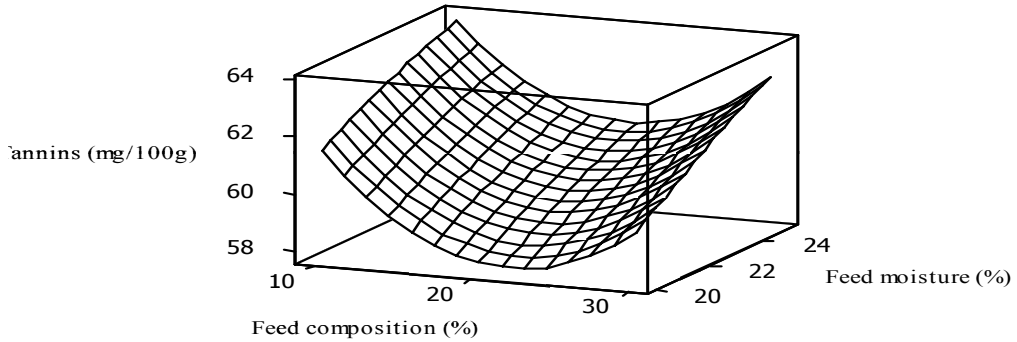

Figure 3: Response surface plot of effect of feed composition and moisture on the residual tannin content of extrudates.

3.4 Phytic acid

The residual phytic acid content of the extrudates ranged from 54.11 to 88.4 mg/100g. The highest residual phytic acid content was found from Run 3 (10:25:120) while the lowest value was from Run 5 (10:20:160). Residual phytic acid content of the extrudates decreased to a minimum at 20% feed cowpea flour addition and thereafter increased with increase in cowpea flour. The interaction effects of feed composition and barrel temperature showed the most influence on the phytic acid content of the extrudates compared to the other independent variables. The residual phytic acid content of the extrudates (54.11 to 88.4 mg/100g) was also significantly ($p<0.05$) lower than that of the raw sorghum-cowpea blends (329.16 to 415.12 mg/100g). A 13-35% reduction in phytate content was observed after extrusion of wheat bran-starch gluten mix (Andersson *et al*., 1981). Singh *et al*. (2007) reported that extrusion hydrolyses phytate to release phosphate molecules. Extrusion of peas and kidney beans resulted in phytate hydrolysis, which explained the higher availability of minerals after high temperature extrusion (Alonso *et al*., 2001). Application of a single technique to reduce antinutrients is often insufficient for effective treatment and so, a combination of treatments such as dehulling and extrusion cooking which were combined in this process may be appropriate. The phytic acid content of cowpea extrudates was significantly ($p<0.05$) influenced by the interaction effects of feed composition and barrel temperature. The coefficient of determination (R^2) was 0.99 with a non-significant lack-of-fit. The observed and predicted values were also in close agreement ($r^2 = 0.99$) suggesting a good fit for the model. The model was therefore found adequate in describing the residual phytic acid of the extrudates. The optimum residual phytic acid content of 72.24 mg/100g was found at 22.26% feed composition, 24.3% feed moisture and barrel temperature of 133.17 ^oC. The response surface plots of residual phytic acid content of extrudates is presented in Figure 4.

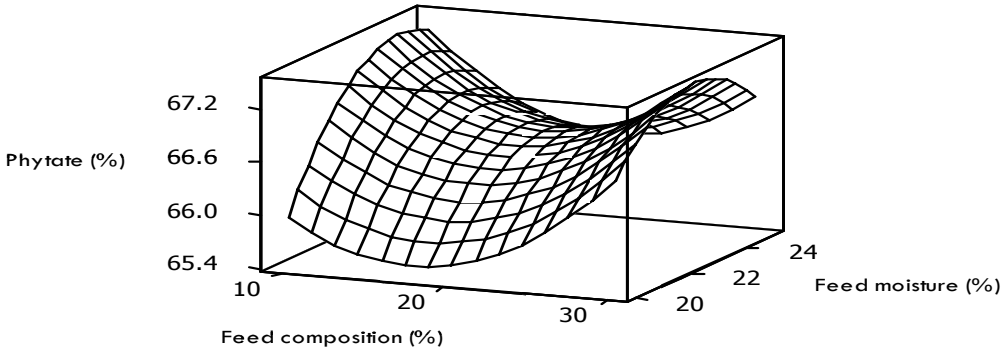

Figure 4: Response surface plot of effect of feed composition and feed moisture on the residual phytate content of extrudates.

4. Conclusion

A breakfast cereals with improved *in vitro* protein digestibility and low phytic acid and polyphenol content were produced from extruded sorghum-cowpea blends. Protein digestibility generally increased as the barrel temperature was increased. The interaction effects of feed moisture and barrel temperature showed the most influence on the residual polyphenol content of the extrudates while the interaction effects of feed composition and barrel temperature showed the most influence on the phytic acid content of the extrudates. The second order polynomial was found appropriate for the prediction of polyphenols, phytic acid and protein solubility and digestibility of the sorghum-cowpea extrudates. A combination of treatments such as dehulling and extrusion cooking may be appropriate in reducing the polyphenols and phytic acid content and thus improving protein

digestibility of sorghum-cowpea extrudates.

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References

Andersson, Y., Hedlund, B., Jonsson, L. and Svensson, S. (1981). Extrusion cooking of a high – fibre cereal product with crisp bread character. *Cereal Chemistry*, 58, 370-374.

Annor, G.A, Sakyi-Dawson, E, Saalia, F.K., Sefa-Dedeh, S., Afoakwa, E.O., Tano-Debrah, K and Budu, A.S. (2009). Response surface methodology for studying the quality characteristics of cowpea (*Vigna unguiculata*) – based tempeh. *Journal of Food Process Engineering,* Pp.1-20

Alonso, R., Rubio, L.A., Muzquiz, M. and Marzo, F. (2001). The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seed meals. *Animal Feed Science and Technology*, 94, 1 -13.

BS (1985). British Standard (812-103.1:1985), Now European Standards EN 933-1 (2012). http://en.wikipedia.org/w/index.php?title=mesh(scale). Accessed 5/03/2013.

Chavan, J.K., Kadam, S.S. and Salunkhe, D.K. (1989). Cowpea. In: Salunkhe, D.K. and Kadam, SS. (eds). *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing, Technology and Utilization,* Vol. II. Boca – Raton FL: CRC Press Inc. Pp. 1 – 21.

Davies, N.T. and Raid, H. (1979). An evaluation of the phytate, zinc, copper, iron and manganese contents of, and zinc availability from soya-based textured vegetable protein meat – substitutes or meat – extenders. *British Journal of Nutrition*, 41, 579.

Egan, H., Kirk, R. S. and Sawyer, R. (1981). *Pearson's Chemical Analysis of Foods*, 8th ed. Pub. Churchill Livingstone.

Fapojuwo, O. O., Maga, J. A. and Jansen, G. R. (1987). Effect of extrusion cooking on *in vitro* protein digestibility of sorghum. *Journal of Food Science*, 52, 218-219.

Filli, K.B., Nkama, I., Abubakar, U.M. and Jideani, V.A. (2010). Influence of extrusion variables on some functional properties of extruded millet-soybean for the manufacture of "Fura": A Nigerian traditional food. *African Journal of Food Science*, 4(6), 342 – 352.

Garcia, - Estepa, R.M., Guerra – Hernandez, E. and Garcia – Villanova, B. (1999). Phytic acid content in milled cereal products and breads. *Food Research International*, 32, 217 – 221.

Gomez, M. H., Waniska, R. D., Rooney, L. W. and Lusas, E. W. (1988). Extrusion cooking of sorghum containing different amounts of amylose. *Journal of Food Science*, 53 (6), 1818-1822.

Gomez, M. I. Obilana, A. B. Martin, D. F., Madzvamuse, M. and Monyo, E. S. (1997). *Manual of Laboratory Procedures for Quality Evaluation of Sorghum and Pearl Millet,* Technical Manual No. 2 (In: En. Abstracts in Fr.) Patancheru 502, 324. Andra Pradesh, India. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Hamaker, B. R. and Bugusu, B. A. (2003). Overviev: Sorghum proteins and food quality. Workshop on the proteins of sorghum and millets; Enhancing nutritional and functional properties for Africa. Pretoria, South Africa. 2nd – 4th April, 2003. http://www.afripro.org.uk/papers.

Hamaker, B. R., Kirleis, A.W., Butler, L.G., Axtell, J.D. and Mertz, E.T. (1987). Improving the *in-vitro* protein digestibility of sorghum with reducing agents. In: *Utilization of Sorghum and Millet*, eds. M.I. Gomez, L.R. House, L.W. Rooney and D.A.V., Dendy, ICRISAT Pub.1992 pp 61 – 62.

Hauck, B.W. (1981). Contol of process variables in extrusion cooking. *Journal of Food Science*, 26, 170-173.

Iwe, M.O., van Zuilichem, D.J., Ngoddy, P.O. and Lammers, W. (2001). Amino acid and protein digestibility index of mixtures of extruded soy and sweet potato flours. *Lebensmittel Wissens-chaft and Technol.,* 34, 71 – 75.

Joglekar, A.M. and May, A.T. (1987). Product excellence through design of experiments. *Cereal Foods World,* 32, 857-868.

Maclean, W.C., Lopez de Romana, G.L., Placko, R.P., Graham, G.G. (1981). Protein quality and digestibility of sorghum in preschool children: Balanced studies and plasma free amino acids. *Journal of Nutrition,* III, 1928 – 1936.

Malaysian Standard (1997). *Animal Feeding Stuffs- Determination of Protein Solubility Index,* Ics: 65, 120. Department of Standards, Malaysia.

Mertz, E.T., Hassen, M.M., Cairns-Whittern, C., Kirleis, A.W., Tu, L. and Axtel, J.D. (1984). Pepsin digestibility of proteins in sorghum and other major cereals. *Proc. Natl. Acad*. Sa. USA 81.

MINITAB 14 (2003). Meet MINITAB Release 14 for Windows Manual. Minitab Inc. ISBN 0-925636-48-7.

Nkama, I. and Gbenyi, D.I. (2002). The Effect of Malting of Millets and Sorghum on the residual Phytates and

Polyphenols in *Dakuwa*- A Nigerian Cereal-Legume Snack Food. *Nigerian Journal of Tropical Agriculture,* 3, 76-84.

NPC (2001). National Planning Commission, Abuja. National Policy on Food and Nutrition in Nigeria.

Nicholson, N.F. (1992*). Processing of Sorghum in Botswana for Foods and Feeds: Problems and Opportunities.* In*: Utilization of Sorghum and Millets.* Eds M.I. Gomez, L.R. House, L.W. Rooney and Dendy, D.A.V. ICRISAT Pub. India. Pp 99 – 101.

Onyango, C, Noetzold, H., Bley, T. and Henle, T. (2004b). Proximate composition and digestibility of fermented and extruded *uji* from maize- finger millet blend. *Swiss Society of Food Science and Technology*, 37, 827- 832.

Pelembe, L.A.M., Erasmus, C., and Taylor, J.R.N. (2002). Development of a protein-rich composite sorghum-cowpea instant porridge by extrusion cooking process. *Lebensmittel Wissenschaft Und-Technologie,* 35, 120 – 127.

Price, M.L. and Butler, L.G. (1977). Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. *Journal of Agricultural Food Chemistry*, 25, 1266 – 1273.

Reddy, R.N., Pierson, M.D., Sathe, S.K. and Salunkhe, D.K. (1985). Dry bean tannins: A review of nutritional implications. *Journal of American Oil Chemical Society*, 62, 541–549.

Reddy, R. N., Pierson, M.D., Sathe, S.K. and Salunkhe, D.K. (1989). *Phytates in Cereals and Legumes.* Boca Raton, F.L.: CRC Press.

Reddy, R.N. and Pierson, M.D. (1994). Reduction in antinutritional and toxic components in plant foods by fermentation. *Food Research International,* 27, 281 – 290.

Rooney, L.W. (1992). *Methods of Processing Sorghum for Livestock***.** In: *Utilization of Sorghum Millets.* ICRISAT Pub. Pp. 167 – 171.

Rizvi, S.N., Mulvancy, S.J. and Sokhncy, A.S. (1995). The combined application of supercritical fluid and extrusion technology. *Trends in Food Science Technology,* 6(7), 232-240.

Salunkhe, D.K., Chavan, J.K. and Kadam, S.S. (1990). *Dietary Tannins, Consequences and Remedies.* Raton, F.L.: CRC Press.

Shinde, G.B. Adsule, R. N. and Kale, A. A. (1991). Effectof dehulling and cooking treatment on phytate phosphorus, polyphenols and trypsin inhibitor activity of cowpea seed. *Industrial Food Packaging*, 36, 63-65.

Singh, S.; Gamlath, S.; and Wakeling, L. (2007). Nutritional aspects of food extrusion: A review. *International Journal of Food Science and Technology*, 42, 916 – 929.

Wu, P., Tian J.C, Walker C.E. and Wang F.C. (2009). Determination of phytic acid in cereals. A brief review. *Tnt. Journal of Food Science and Technology,* l44, 1671-1676.

Zasypkin, D.V. and tung – Ching, L. (1998). Extrusion of soybean and wheat flour as affected by moisture content. *Journal of Food Science*, 63(6), 1058 – 1061.