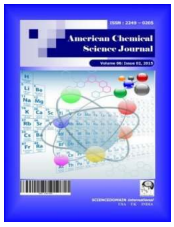

American Chemical Science Journal
17(1): 1-14, 2016, Article no.ACSJ.28565
ISSN: 2249-0205

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Residual Polyphenols, Phytate and *In-vitro* Protein Digestibility of Extruded Sorghum-Bambara Groundnut Blends

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DIG and ND designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author IN managed the analyses of the study. Author MHB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACSJ/2016/28565
Editor(s):
(1) T. P. West, Department of Chemistry, Texas A&M University-Commerce, USA.
Reviewers:
(1) Fatema Hossain Brishti, University Putra Malaysia, Malaysia.
(2) Anonymous, Chiang Mai University, Thailand.
(3) Nadzirah Kamarul Zaman, Universiti Teknologi MARA (UiTM), Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history/16282

Original Research Article

Received 26th July 2016
Accepted 1st September 2016
Published 21st September 2016

ABSTRACT

Aim: This study was designed and implemented to produce instant breakfast cereals from the blends of sorghum and Bambara groundnut flour through extrusion cooking as a strategy to reduce phytic acid and polyphenol and improve the protein digestibility and solubility.
Methodology: In this study, different blends of Bambara groundnut (10%, 20% and 30%) and sorghum flour were extruded at 20%, 22.5% and 25% moisture levels and 120℃, 140℃ and 160℃ barrel temperatures using a single-screw extruder. Response surface methodology with

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central composite face-centered design (CCFD) was used to model the residual phytic acid and polyphenols, protein solubility and protein digestibility of the extrudates.
Results: 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 0.96, 0.83, 0.95 and 0.94 for polyphenols, phytic acid, protein solubility and protein digestibility respectively with non-significant lack of fit in all cases. Barrel temperature had the most effect on the responses.
Conclusion: Extrusion cooking in combination with dehulling significantly increased the protein digestibility of sorghum-Bambara groundnut extrudates. The second order polynomial was found appropriate for the prediction of polyphenols, phytic acid and protein digestibility of the sorghum-Bambara groundnut extrudates. The optimum levels of the response variables attainable were 11.32%, 70.0%, 48 mg/100 g and 63.11 mg/100 g respectively for protein solubility, digestibility, residual polyphenols and phytate content.

Keywords: Response surface; digestibility; polyphenols; tannins; extrusion.

NOMENCLATURES

RSM : Response Surface Methodology
CCFD : Central Composite Face-centered Design
CCD : Central Composite Design

1. INTRODUCTION

Antinutritional factors such as polyphenols (tannins) have been reported to be one of the factors affecting the selection and utilization of sorghum for food [1,2]. The tannins in sorghum have been found to form complexes with proteins, reducing their biological value. Hamaker et al. [3], Maclean et al. [4] and Mertz et al. [5], 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 processing, thereby creating less-digestible proteins.

It has been argued [2] 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 are grouped into three: phenolic acids, flavonoids, and tannins. All sorghum varieties contain phenolic acids and most contain flavonoids. Brown-seeded and bird resistant sorghum varieties contains condensed tannins, while varieties without pigmented testa do not have condensed tannins, and their nutritional value is 95% that of maize [2]. The resistance of red sorghum to both bird and fungi attack makes it attractive to Nigerian farmers, but Nigeria is characterized by poor food processing and preservation technology. This raises concerns on how best to reduce the polyphenols in high tannin sorghums to acceptable levels through alternative processing technologies.

Tannins and phytates have been found to reduce both protein and mineral bioavailability in human diet [6,7]. 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. They are reported to interact with proteins (both enzyme and non-enzyme proteins) to form complexes, resulting in inactivation of digestive enzymes and protein insolubility [8,7]. The result is lowered feed efficiency, growth depression and decreased mineral 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 [9]. Phytate on the other hand 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+} [10].

Elimination of antinutritional factors in food crops can either be achieved by selecting genotypes with low levels of these factors or through postharvest processing such as boiling, germination, roasting, leaching, fermentation, extraction and extrusion [11]. Extrusion cooking of sorghum is reported to have improved its digestibility significantly [12]. Alteration of pH before extrusion further improved digestibility. Gomez et al. [13] extruded three sorghum varieties containing different amounts of amylose and found out that different amylose/amylopectin ratios in the sorghum meal did not affect the *in vitro* protein digestibility of the extrudates;

however, digestibility increased as extrusion moisture level decreased. Extrusion cooking technology therefore with its numerous advantages over traditional and conventional food processing methods is one of the most versatile and energy efficient processes currently contributing solutions to world hunger and nutritional problems and improvement of quality of processed foods [14,15].

It has been reported [16,17,18] that the one-variable-at-a-time method of experimentation is inappropriate in many circumstances, especially where the process involves multiple variables. Response surface methodology (RSM) is useful in situations such as extrusion cooking, where several input variables potentially influence some performance measure or quality characteristics of the process. RSM explores the space of the process or independent variables and uses empirical statistical modeling to develop an appropriate approximating relationship between the yield and the process variables, and optimization methods for finding the values of the process variables that produce desirable values of the response [19]. RSM is a critical technology in developing or testing new processes, optimizing their performance, including reduction of variability and improved process and product performance [19]. The RSM permits definition of empirical models such as linear, linear with two factor interaction or quadratic polynomials which describe accurately how responses behave at all values of the studied variables in the experimental region [20]. It is used to model and analyze problems whose designed responses are influenced by many variables [21] as well as optimize processes and products [19].

The Central Composite Design (CCD) is a response surface design where a process of sequential experiments using independent variables lead to an optimum response [22]. It is form of a general 2^k factorial design where k denotes the number of factors and the number 2 denotes two levels (high or low) of the factors. The difference between a 2^k factorial design and CCD is that the CCD includes the central point. This is advantageous in order to verify the response near the central condition. Also the CCD takes into account the changes in the response due to interaction of the factors and the quadratic effects [22].

Sorghum is an important component of Nigerian diet. Most households spend more than 75 % of their income on food alone [23] and minimally process sorghum before eating, thus creating room for high prevalence of nutritional disorders related to high intake of anti-nutritional factors and food/nutrition insecurity. Many Nigerians do not have access to animal protein because of its high cost. The utilization of extrusion cooking technology and supplementation of sorghum flour with plant protein from Bambara groundnut flour in the production of instant, high energy and protein breakfast cereal with good storage life and low microbial load is likely to increase protein quantity and quality of common food products.

This study was designed and implemented to produce instant breakfast cereals from the blends of sorghum and Bambara groundnut flour through extrusion cooking as a strategy to reduce phytic acid and polyphenols and improve the protein digestibility and solubility. This will enhance the utilization of sorghum for the production of good quality value-added complementary foods that will contribute to the reduction of protein-energy-malnutrition in Nigeria and other countries having similar food habits.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Procurement of raw materials

The red sorghum variety (*Chakalari red*) was obtained from Maiduguri Monday market, Borno State, while Bambara groundnut (Cream-coloured variety) was purchased from Mubi main market, Adamawa State, Nigeria.

2.1.2 Production of sorghum flour

Fifteen kilogram (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 clean tap water in plastic basins and sun dried on mats for 2 days at ambient temperature of 38±2^{o}C and relative humidity of 27.58% to 12-14% 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 [24] before packing in polythene bags for further use as earlier reported [25].

2.1.3 Production of Bambara groundnut flour

The traditional method of preparation of Bambara groundnut flour was adopted. In this process, the Bambara groundnut was first washed with clean water and then dried in a Chirana convection air oven (Model: HS 201A, Hungary) to 12-14% moisture. This was then milled using an attrition mill (Imex GX 160, Japan) and sieved to pass mesh number 25 [24] to obtain the flour.

2.1.4 Blending of sorghum flour with Bambara groundnut flour and moisture adjustment

Sorghum flour was blended with Bambara groundnut flour in varying proportions (10%, 20%, and 30% Bambara groundnut). The individual moisture contents of the Bambara groundnut and sorghum flours were determined (on dry weight basis) using the hot air oven method [26] and the total moisture of the blends adjusted to the desired level according to Zasypkin and Tung-Ching [27]. The blends were mixed using a laboratory mixer (Hobert, Model: A200) and the moisture allowed to equilibrate for 1 h before extrusion.

2.2 Methods

2.2.1 Protein solubility test

The Malaysian Standard method [28] reported in Annor et al. [29] 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 g 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 15 mL supernatant was transferred into two Kjeldahl tubes for duplicate analysis (this gives 0.3 g 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 was the soluble protein fraction (from supernatant) expressed as a percentage of the total protein in the sample.

$$Protein\ Solubility\ (\%) = \frac{\text{Protein in filterate}}{\text{Total protein in sample}}\ X\ 100$$

2.2.2 Protein digestibility test

Protein digestibility was determined according to the procedures of Onyango et al. [30]. 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 hr in a water bath at 37℃, 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℃ for 3 hr. The dried residue was analyzed for nitrogen using Kjeldahl method.

$$In\ vitro\ Protein\ Digestibility\ (\%) = \frac{\text{CP1} - \text{CP2}}{\text{CP1}}$$

Where:

CP1 = Total protein of extrudate
CP2 = Total protein after digestion with pepsin

2.2.3 Phytic acid determination

Phytate (phytic acid) content of samples was determined by the method described by Davies and Reid [31]. 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℃ 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 [32].

2.2.4 Polyphenol determination

The Prussian blue assay for the determination of total polyphenols [33] was used. Ground samples (0.035 g 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 of catechin hydrate prepared according to Gomez et al. [34] 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, and 1.0 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.

2.3 Experimental Design

RSM and Central Composite Face-Centered Design (CCFD) were used to design the experiment layout and study the possible effects of the independent variables on the tannin, phytic acid, protein solubility and digestibility using MINITAB 14 statistical software [35]. Table 1 shows the process variables and their levels used in the design. The experimental matrix based on CCFD is presented 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 [29] of the form:

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

Where X_1, X_2, and X_3 are feed composition (Bambara groundnut flour), feed moisture and barrel temperature, respectively; b_0 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.

2.4 The Extrusion Cooking Process

Extrusion cooking was carried out using a single screw extruder (Brabender Duisburg DCE-330), equipped with a variable speed DC drive unit and strain gauge type torque meter. The various formulations were fed manually through a screw operated conical hopper which is mounted vertically above the end of the extruder and equipped with a screw which was adjusted to 139 rpm. The feeds 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 adjusted 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.

2.5 Statistical Analysis

MINITAB version 14 statistical analysis software [35] was used for the statistical analysis. Multiple regression analysis was conducted and results fitted to second-order polynomial equation to develop a model equation that will establish the relationship between the independent and response variables. Analysis of variance (ANOVA) was conducted on the data to establish statistical level of significance of the model, while coefficient of determination test (R^2), test of lack of fit and residual analysis were conducted to determine the adequacy of the fitted models. Correlation analysis was used to test the relationship between the predicted and observed values, while numerical optimization and interactive graphs (3D) were plotted and used to locate the optimum levels of the various input variables and responses.

Table 1. Process variables and their levels used in the central composite face centered design (CCFC)

Independent variables	Symbol	Unit	Coded levels of independent variables		
			-1	0	+1
Feed composition	X_1	g/100 g	10	20	30
Feed moisture content	X_2	g/100 g	20	22.5	25
Extrusion temperature	X_3	℃	120	140	160

Table 2. Central composite face-centered (CCFC) design matrix for independent variables in their coded and natural forms

Experimental runs	Independent variables in its natural and coded levels		
	X_1	X_2	X_2
1	10 (-1)	20 (-1)	120 (-1)
2	30 (+1)	20 (-1)	120 (-1)
3	10 (-1)	25 (+1)	120 (-1)
4	30 (+1)	25 (+1)	120 (-1)
5	10 (-1)	20 (-1)	160 (+1)
6	30 (+1)	20 (-1)	160 (+1)
7	10 (-1)	25 (+1)	160 (+1)
8	30 (+1)	25 (+1)	160 (+1)
9	10 (-1)	22.5 (0)	140 (0)
10	30 (+1)	22.5 (0)	140 (0)
11	20 (0)	20 (-1)	140 (0)
12	20 (0)	25 (+1)	140 (0)
13	20 (0)	22.5 (0)	120 (-1)
14	20 (0)	22.5 (0)	160 (+1)
15	20 (0)	22.5 (0)	140 (0)
16	20 (0)	22.5 (0)	140 (0)
17	20 (0)	22.5 (0)	140 (0)
18	20 (0)	22.5 (0)	140 (0)
19	20 (0)	22.5 (0)	140 (0)
20	20 (0)	22.5 (0)	140 (0)

X_1 = Bambara groundnut composition, X_2 = Feed moisture level and X_3 = Barrel temperature

3. RESULTS AND DISCUSSION

3.1 Protein Digestibility and Solubility of Sorghum-Bambara Groundnut Extrudates

Results of protein solubility, digestibility and residual tannins and phytate levels are presented in Table 3. Protein digestibility values were lowest at Run 9 corresponding to 10% feed composition, 22.5% feed moisture and 140^{o}C barrel temperature, with a value of 67.50% and highest at Run 2 (30% feed composition, 20% feed moisture and 120℃ barrel temperature) with a value of 73.50%. Protein digestibility of raw sorghum flour was 44.22%. Protein digestibility increased significantly ($p<0.01$) as the extrusion temperature increased. High temperatures in combination with high screw speed (200 rpm in this case) may have caused more severe denaturation and disruption of the protein matrices in sorghum thus making them susceptible to enzyme digestion. These results are in agreement with the reported digestibility values of 87.90% to 89.70% for cereal-legume extruded foods [36,37,38]. Pepsin digestibility of decorticated, extruded sorghum flour was found to be 79.0% by Mertz et al. [5]. This was much higher than the values for decorticated and non-extruded sorghum flour (56.80%) from the same cultivated variety. This shows that extrusion cooking of sorghum improves its *in vitro* protein digestibility. Sorghum generally has poor protein digestibility and must be properly processed to obtain its optimal nutritional potentials. MacLean et al. [4,38] feeding the same decorticated extruded cooked sorghum flour to malnourished children obtained an average value of 81% protein digestibility. The improvement of *in vitro* protein digestibility through extrusion cooking may probably be by promoting thermally induced cross-links among sub-units of proteins, which exposes the enzyme active sites of the protein.

Protein solubility values for Bambara groundnut extrudates ranged from 10.15% to 15.95%. The lowest value was found at Run 7 (10% feed composition, 25% feed moisture and 160℃ barrel temperature), while the highest value was located at Run 4 (30% feed composition, 25% feed moisture and 120℃ barrel temperature). Protein solubility of raw sorghum flour was 2.83%. Protein solubility is impaired by high temperature due to denaturation which may be responsible for the higher solubility observed at lower temperatures (120℃). Protein solubility of the sorghum-Bambara groundnut extrudates was affected by the quadratic effects of feed composition only. It was not affected by the linear or interaction terms of the model equation. The coefficient of determination was 0.95. The lack of fit of the model was not significant ($p<0.05$). These suggest that the model adequately described the protein solubility of the sorghum-Bambara groundnut extrudates.

3.2 Residual Phytic Acid and Polyphenol Content of Sorghum-Bambara Groundnut Extrudates

The residual phytic acid content of Bambara groundnut extrudates varied from 49.07 to 73.19 mg/ 100 g. The lowest value for phytic acid content of Bambara groundnut extrudates was found at Run 6 (30% feed composition, 20% feed moisture and 160℃ barrel temperature) while the highest value was at Run 1(10% feed composition, 20% feed moisture and 120℃ barrel temperature). Samples extruded at lower temperatures tended to have higher residual phytic acid and polyphenol content than those extruded at higher temperature. Extrusion at higher temperatures may have caused a change in the original structure of phytic acid thus

reducing its concentration in the extrudates. Phytic acid content of raw dehulled sorghum flour was 348.12 mg/100 g. Bambara groundnut extrudates had residual polyphenol values ranging from 35.48 to 53.5 mg/100 g. The lowest value was located at Run 14(20% feed composition, 22.5% feed moisture and 160℃ barrel temperature) while the highest value was located at Run 4 (30% feed composition, 25% feed moisture, 120℃ barrel temperature). The polyphenol content of raw sorghum flour after dehulling was 286.3 mg/100 g. Polyphenols are the secondary plant metabolites and are distributed ubiquitously within plant foods (vegetables, cereals, legumes, fruits, nuts etc.) and beverages (tea, wine, cocoa etc). Their levels vary greatly even within cultivars of the same species. Environmental factors such as light, germination, degree of ripeness, variety, processing and storage and genetic factors influence their levels [39,40,41].

3.3 Fitting Regression Models

The regression equations describing the effects of the independent variables on the total tannin, phytate contents and protein solubility and digestibility are given in Table 4.The coefficients in the regression equations can be used to examine the statistical significance of each of the term in relation to each other when used with coded value [36]. The goodness-of-fit of the models were determined by considering the coefficients of determination (R^2). In this case, the R^2 values were 94.65%, 94.38%, 96.06% and 83.41% respectively for protein solubility, protein digestibility, tannin and phytate. This indicated that the sample variations were 94.65%, 94.38%, 96.06% and 83.41% respectively attributed to the effects of the independent variables and only 5.35%, 5.62%, 3.94% and 16.59% of the total variation could not be explained by the models. However, based on the results of R^2 (Table 4), which ranges between 83.41% and 96.09%, it can be stated that the models fitted could represent the true state of the relationship between the independent and response variables in their natural form. Danbaba et al. [42] reported that R^2 close to 100% ensure satisfactory fitting of predictive regression model to real system.

The adjusted coefficient of determination (R^2_{adj}) values of 0.93, 0.79, 0.9 and 0.9 for tannin, phytate, protein solubility and digestibility (Table 5) were also satisfactory, confirming the significance of the models. Zaibunnisa et al. [43] and Danbaba et al. [42] suggested that R^2 value

Table 3. Protein solubility and digestibility, tannin and phytate of sorghum-Bambara groundnut extruded instant breakfast cereals

Runs	BF:FM:ET	Protein solubility (%)	Protein digestibility (%)	Tannins (mg/100 g)	Phytate (mg/100 g)
1.	10:20:120	10.82	67.57	48.87	73.19
2.	30:20:120	15.09	73.50	49.75	69.53
3.	10:25:120	10.73	67.78	48.89	70.35
4.	30:25:120	15.95	72.65	53.50	70.16
5.	10:20:160	10.93	68.25	39.41	50.37
6.	30:20:160	14.97	72.20	39.76	49.07
7.	10:25:160	10.15	69.16	38.48	49.14
8.	30:25:160	14.91	73.20	38.76	50.74
9.	10:22.5:140	11.95	67.50	46.17	59.97
10.	30:22.5:140	14.65	71.71	46.63	61.29
11.	20:20:140	11.03	68.85	44.43	59.96
12.	20:25:140	10.92	68.82	46.71	60.92
13.	20:22.5:120	12.80	70.05	51.21	52.20
14.	20:22.5:160	10.70	70.05	35.48	49.55
15.	20:22.5:140	11.94	68.80	36.27	59.81
16.	20:22.5:140	12.15	69.76	47.35	60.51
17.	20:22.5:140	11.72	69.30	46.90	59.79
18.	20:22.5:140	12.15	68.12	46.37	61.65
19.	20:22.5:140	11.48	68.05	46.93	60.07
20.	20:22.5:140	12.1	67.54	46.24	60.42

BF = Bambara groundnut flour, FM = Feed moisture, BT = Barrel temperature

Table 4. Predictive regression models for the relationship between the independent and response variables

Response variables	Regression model	R^2
Protein solubility	$42.3438 – 0.5788X_1 + 5.2251X_2 + 0.0063X_3 + 0.0166X_1^2 – 0.1075X_2^2 + 0.0003X_3^2 + 0.0084X_1X_2 – 0.0005X_1X_3 – 0.004X_2X_3$	0.9465
Protein digestibility	$132.735 + 0.2915X_1 – 0.4731X_2 – 0.9277X_3 + 0.0073X_1^2 – 0.0062X_2^2 + 0.003X_3^2 – 0.0049X_1X_2 – 0.0018X_1X_3 +0.0064X_2X_3$	0.9438
Tannin	$-83.0686 – 0.203X_1 + 2.6207X_2 + 1.7212X_3 + 0.007096X_1^2 – 0.0184X_2^2 – 0.0059X_3^2 + 0.0183X_1X_2 – 0.0031X_1X_3 – 0.0142X_2X_3$	0.9609
Phytate	$148.5366 – 2.5301X_1 – 5.9335X_2 + 3.6920X_3 + 0.0357X_1^2 + 0.5408X_2^2 – 0.0155X_3^2 + 0.0318X_1X_2 + 0.0026X_1X_3 + 0.0067X_2X_3$	0.8341

Table 5. Regression coefficients for protein solubility and digestibility, residual tannins and phytic acid of extrudates

Coefficients	Response variables			
	Tannins	Phytate	Solubility	Digestibility
Linear				
b_o	-83.069	148.537	-42.344	132.735
b_1	-0.203	-2.5301	-0.5788	0.2915
b_2	2.621	-25.934	5.2251	-0.4731
b_3	1.721**	3.6920	0.0063	-0.9277**
Quadratic				
b_{11}	0.0071	0.0357	0.0166**	0.0073
b_{22}	-0.0184	0.0357	-0.1075	-0.0062
b_{33}	-0.0059**	-0.0155*	0.0003	0.003**
Interaction				
b_{12}	0.0183	0.0318	0.0084	-0.0049
b_{13}	-0.0031	0.0026	-0.0004	-0.0018
b_{23}	-0.0143	0.0066	-0.004	0.0064
R^2	0.9609	0.8341	0.9463	0.9438
Adjusted R^2	0.9258	0.7848	0.8979	0.8931
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 = Barrel temperature; * Significant at p≤0.05, and **p≤0.01 respectively, Ns = Not significant*

should be at least 80% to have good fit of a regression model. F-test was used to determine whether the lack-of-fit test was significant or not. The results of this study indicated non-significant ($p<0.05$) lack-of-fit in all the variables. The results of this study revealed that the model for all the response variables were adequate to explain the variability in responses. The regression equations demonstrate that polyphenols, phytate, *in-vitro* protein digestibility and solubility were empirical functions of the test variables as presented in Table 4.

Figs. 1a-d show the residual plots indicating the correlation between experimental versus predicted values for protein solubility, digestibility, tannin and phytate respectively. From the residual plots it is clear that, the observed data were close to the predicted values calculated from the model. The correlation coefficients of 0.944, 0.923, 0.983, and 0.834 observed between the predicted and actual values for the response variables are evidence that the regression model can represent the experimental data in its natural state. It could also be observed that the points on the graph were reasonably distributed near a straight line indicating that the underlying regression analysis assumption of normality in this study was appropriate and therefore validate the models fitted.

3.4 Locating Optimum Levels of Process Variables Using 3D Graphs

The 3D graphic representations of response surfaces shown in Fig. 2 help to visualize the effects of feed composition versus extrusion

temperature (a), and feed composition versus feed moisture content (b) on the protein solubility of sorghum-Bambara groundnut extrudates.

The protein solubility of the extrudates increased as the feed composition was increased. Increases in feed composition and extrusion temperature resulted in decreases in the protein solubility of the extrudates. Feed moisture and extrusion temperature had less impact on the protein solubility of the extrudates.

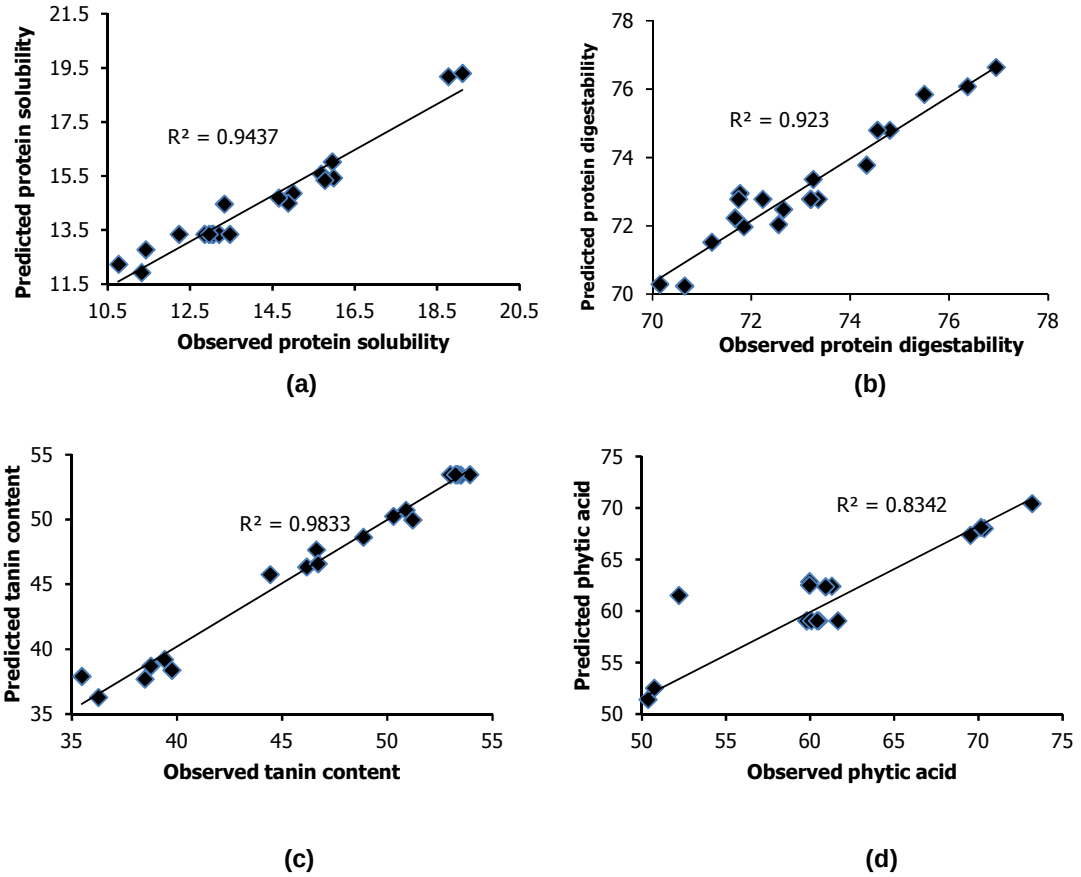


Fig. 1. Residual graph of predicted versus observed values of the response variables with their correlation coefficients

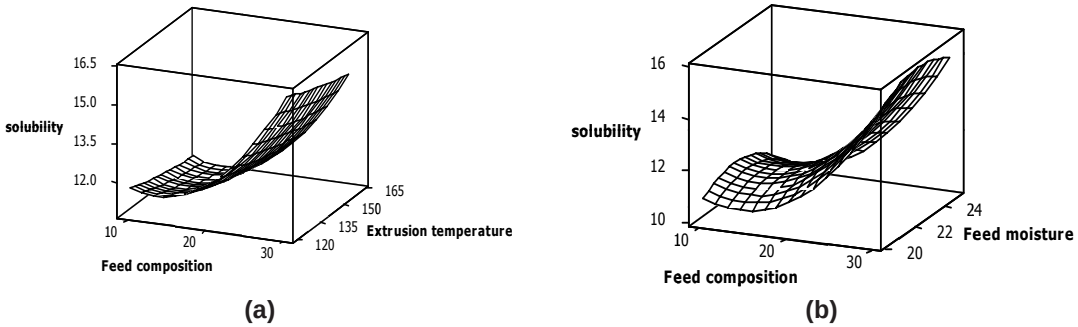


Fig. 2. Effect of feed composition versus extrusion temperature (a) and feed composition versus feed moisture (b) on the protein solubility of sorhum-Bambara groundnut extrudate

The response surface plots of protein digestibility of sorghum-Bambara groundnut-extrudates is presented in Fig. 3. The protein digestibility of the extrudates increased with increase in the Bambara groundnut flour fraction of the feed and increase in extrusion temperature. Feed moisture had non-significant impact on the protein digestibility of the sorghum-Bambara groundnut-extrudates.

Fig. 4 shows the response surface plots of residual phytic acid content of sorghum-Bambara groundnut extrudates. An increase in the extrusion temperature led to a significant decline in the phytic acid content of the extrudates. Increasing the Bambara groundnut flour (up to 20%) along with feed moisture (up to 22%) produced an increase in the phytic acid content of the extrudates (Fig. 4e).

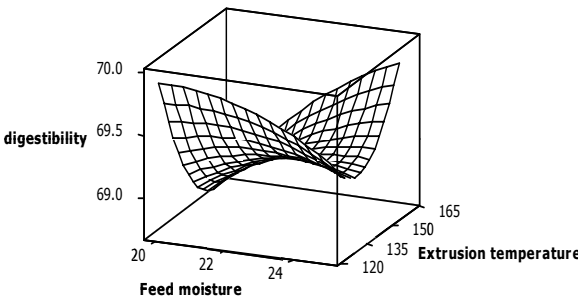
Response surface 3D plot of the residual polyphenol content of sorghum-Bambara groundnut extrudates is shown in Fig. 4f. Increasing the extrusion temperature caused a decline in the polyphyenol content of the extudates. Feed composition, when increased along with feed moisture produced a significant increase in the residual polyphenol content of the extrudates. Extrusion temperature showed the most effect in reducing the polyphenol content of the extrudates.

3.5 Numerical Optimization

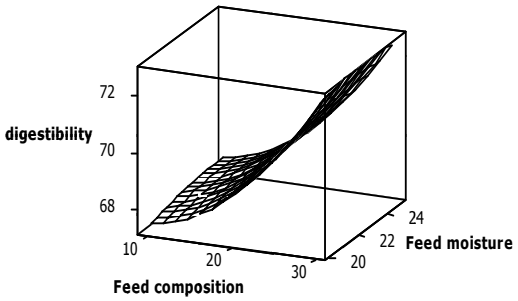
In this process, the targets were defined thus:

- Minimize the levels of phytate and tannins
- Maximize protein solubility and digestibility

In order to optimize the process conditions and obtain optimum levels of the responses, the first partial derivative of the regression model developed was equated to zero. Under the conditions outlined in Table 6 for feed composition, moisture level and extrusion temperature, the optimum levels for the responses were 11.32%, 70.0%, 48.0 mg/100 g and 63.11 mg/100 g respectively for protein solubility, digestibility, and tannins and phytate levels.

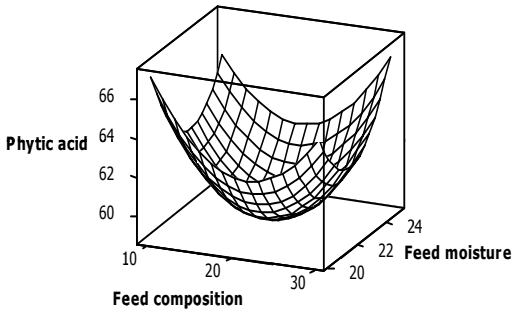


(c)

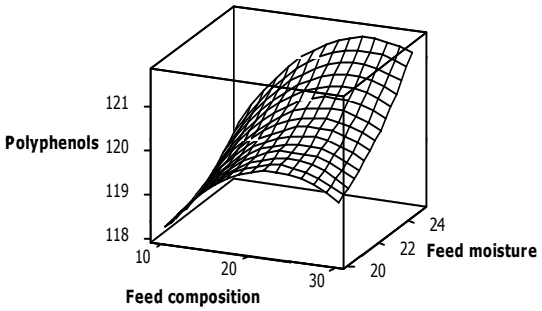


(d)

Fig. 3. Effect of feed moisture versus extrusion temperature (c) and feed composition versus feed moisture (d) on protein digestibility of sorghum-Bambara groundnut extrudates



(e)



(f)

Fig. 4. Effect of feed composition versus feed moisture on the phytic acid (e) and polyphenols contents (f) of sorghum-Bambara groundnut extrudates

Table 6. Optimization of protein solubility and digestibility, residual tannins and phytate of sorghum-Bambara groundnut extrudates

Responses	BF (%)	FM (%)	BT (^{o}C)	Optimum level
Solubility	10	24.80	123.6	11.32%
Digestibility	22.26	24.24	125.5	70.0%
Tannins	10	20	129	48.0 mg/100 g
Phytate	10	20.4	124	63.11 mg/100 g

BF = Bambara groundnut flour, FM = Feed moisture, BT = Barrel temperature

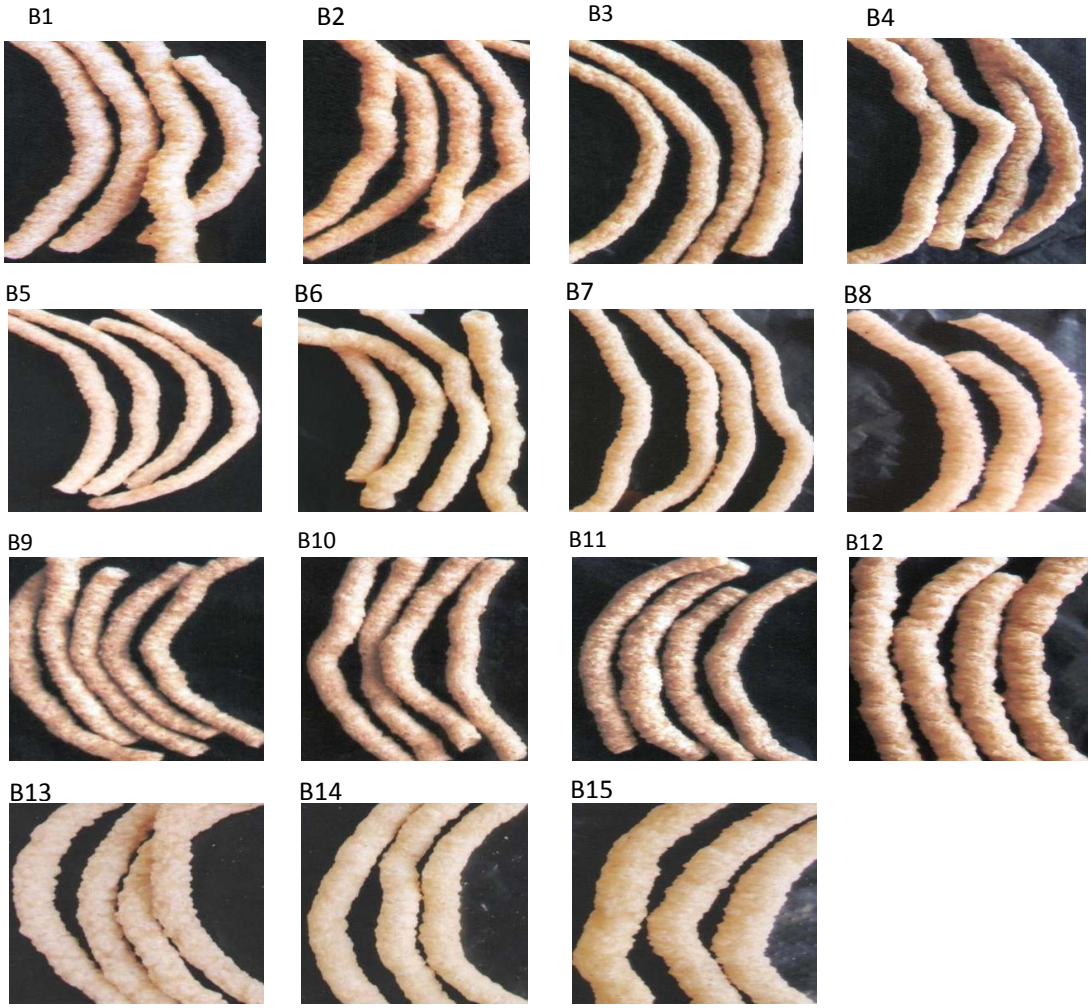


Plate B1-B15. Photographic image of extrudates as affected by varying extrusion conditions

3.6 Photographic Responses of Sorghum-Bambara Groundnut Extrudates

Plates B1 to B15 are photographic responses of sorghum-Bambara groundnut extrudates. Visually, the highest expansion was observed on plate B15 (30% Bambara groundnut, 25% moisture at 160℃) followed by B12 (10% Bambara groundnut, 25% moisture at 160^{o}C) and B8 (20% Bambara groundnut, 22.5% moisture at 140℃) in that order. The lowest expansion was observed on plate B5 (30% Bambara groundnut, 25% moisture at 120^{o}C) followed by sample B3 (20% Bambara groundnut, 22.5% moisture at 120℃), B7 (20% Bambara groundnut, 20% moisture at 140℃), B9 (20% Bambara groundnut, 25% moisture at

140℃) and B10 (30% Bambara groundnut, 22.5% moisture at 140℃) respectively.

To fully appreciate the physical changes in extruded products as results of varying process conditions and its applications as a quality attributes in extruded products, it is necessary to carefully observe the surface physical characteristics of the extrudates [44]. It is evident from this photographs that extrusion variables significantly affected cooking and subsequently expansion of the extrudates which may be responsible for varying solubility and digestibility of protein and even the levels of antinutritional factors. This was also reported by Filli et al. [45,46] in extrudates produced from millet and some common legumes during the production of *Fura*, a Nigerian traditional porridge.

4. CONCLUSION

Extrusion cooking in combination with dehulling, significantly ($p<0.01$) increased the protein digestibility of extrudates (67.57 to 73.50%) compared to that of raw sorghum (44.22%) and Bambara groundnut (68.92%) flours. Increasing the barrel temperature caused a reduction in protein solubility, residual polyphenols and phytic acid but increased *in vitro* digestibility of the extrudates which is desirable for consumers of the breakfast cereal. The coefficients of determination (R^2) were 0.96, 0.83, 0.95 and 0.94 for polyphenols, phytic acid, protein solubility and digestibility respectively with non-significant lack-of-fit for all responses. The second order polynomial was found appropriate for the prediction of residual polyphenols, phytic acid and protein digestibility of the sorghum-Bambara groundnut extrudates. The optimum levels of the response variables attainable were 11.32%, 70.0%, 48.0 mg/100 g and 63.11 mg/ 100 g respectively for protein solubility, digestibility, residual polyphenols and phytic acid content.

ACKNOWLEDGEMENT

This work was funded in part from the fellowship award of The Federal Polytechnic Mubi to David I. Gbenyi and the World Bank Assisted Science and Technology Education Post-Basic (STEP-B) Project (Credit No: 4304 UNI) of The Federal Polytechnic Mubi, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI: 10.5897/ajfs11.169

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