

Functional properties, *in vitro* protein digestibility and mineral contents of extruded flour developed from maize-cassava leaf composites

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ABSTRACT: Application of extrusion cooking technology in product development is getting a high priority due to its effectiveness in quality improvement. Response surface methodology has been used extensively to optimize extrusion variables. The objective of this study was to evaluate the functional properties, *in vitro* protein digestibility and mineral contents of maize-cassava leaf composite extruded instant porridge flour using response surface methodology. Box-Benkhen design was used for formulation and optimization of the process variables. Extrusion variables; extrusion temperature (80°C – 120°C), feed composition (cassava leaf flour proportion) (5% -15%) and feed moisture (14% - 18%) were used as input variables whereas, water absorption index (WAI), water solubility index (WSI), *in vitro* protein digestibility (IVPD), and minerals were used as responses. Results obtained showed that, WAI and WSI increased significantly ($p < 0.05$) as a result of increase in extrusion temperature and feed composition. WSI showed a significant ($p < 0.05$) positive correlation with IVPD and mineral contents. IVPD was significantly ($p < 0.05$) improved by extrusion temperature and feed composition. Calcium, potassium, magnesium and iron contents were significantly ($p < 0.001$) increased as a result of increase in feed composition. Supplementation of cassava leaf flour during extrusion of maize based products improved its functional properties, IVPD and mineral contents, hence suitable for formulation of nutritious extruded food products.

Keywords: Cassava leaf, extrusion cooking, instant porridge flour, optimization, response surface methodology.

INTRODUCTION

The acceptance of cereal flours by consumers are dependent on its functional properties and the degree of starch gelatinization (Adedeji et al., 2014). Maize (*Zea mays*) grains are used in the production of several traditional foods in developing countries (Mbata et al., 2009). Unfortunately, it lacks some micro-nutrients and its protein digestibility is low (Mbata et al., 2009). In order to alleviate the ever-increasing problems of malnutrition in developing countries, there is a need for supplementation of popularly consumed low-protein staple foods like maize flour with inexpensive locally available sources of plant proteins such as cassava leaves. Cassava leaves are rich in proteins and minerals such as calcium, potassium and

magnesium (Latif and Müller, 2015).

Maize can be processed/supplemented with cassava leaves for product diversity and broader acceptability. Extrusion cooking technology which is used in the food processing industry is attracting global attention due to its versatility and effectiveness in producing shelf-stable products. Extrusion is one among the most commercially successful technologies, escalating its demand in the diverse fields of food processing and digital food marketing (3-D printed food), and food packaging (Prabha et al., 2021), which can be deployed for maize-cassava flour product processing. In extrusion process, raw ingredients are fed into the extruder barrel through the feed hopper

and the screw conveys the food along with it, then products come out in a different diameter of die. Recent advancements in extrusion technology have made it possible to control the process parameters so as to achieve the desired characteristics of the final product (Shah et al., 2021). Product quality can vary considerably depending on the extruder type, screw configuration, feed moisture, temperature profile in the barrel, screw speed, feed composition, feed rate and die profile (Ding et al., 2006).

Response surface methodology consists of a group of mathematical and statistical techniques used in the development of an adequate relationship between a response of interest and a number of factors (Khuri and Mukhopadhyay, 2010). It is a powerful tool to optimize the process parameters in manufacturing processes of various products (Kim et al., 2020). Response surface plots are used to understand the effect of process parameters on responses. The process parameters are optimized using desirability approach of response surface methodology and confirmed by conducting confirmation tests (Chelladurai et al., 2020).

The objective of this study was to evaluate functional properties, *in vitro* protein digestibility and mineral contents of maize-cassava leaf composite instant porridge flour. Response surface methodology with Box-Behnken design was used for formulation and optimization of process variables to achieve the required values of the response variables. Extrusion temperature, feed composition and feed moisture were considered as process variables and water absorption index, water solubility index, *in vitro* protein digestibility, calcium, potassium, magnesium and iron contents were used as response variables.

MATERIALS AND METHODS

Sample collection and preparation

Maize (BH 543) variety (25 kg) was collected from Hawassa Agricultural Research Centre, Southern Ethiopia. The grains were sorted and cleaned to remove foreign matter. The dried kernels were milled into flour using a laboratory disk miller (Alvan Blanch, Britain). The flour was sieved using 100 µm mesh size and packed in polyethylene bag until the extrusion process was carried out. Cassava leaves (*Kello* variety) were collected from *Areka* Research Center located in Wolayta Zone of Southern Ethiopia. To detoxify the cyanides in the cassava leaves, the leaves were fermented according to the methodology by Hawashi et al. (2019), with slight modifications. Chopped cassava leaf (10 kg) was fermented using *Lactobacillus plantarum* (previously isolated from orange juice) at a concentration of 7 mL of 1×10^6 cells/mL and 1:0.5 ratio of cassava leaf to distilled water under incubation temperature of 37°C for 48 hours. Accordingly, the cyanide content of cassava leaf before and after fermentation was 148.77 and 4.21 mg/kg,

respectively. The fermented samples were dried using oven (Binder, Germany) at 60°C for 8 hours. Then, the dried cassava leaf was milled using high speed multi-function comminutor (RRH, 400A) and the flour was packed in polyethylene bag until the extrusion process was conducted.

Maize-cassava leaf composite flour preparation

Three (3) formulations were prepared which contained cassava leaf flour ranging from 5 to 15% as shown on the experimental layout in Table 1. The composites of maize and cassava leaf flour were mixed using a blender. The initial moisture content of the blends were analyzed by drying in oven at 105°C until constant weight was reached using official AOAC Method 925.09 (2005). The initial average moisture content of composite 1 containing 5% cassava leaf flour was 10.94%, while composite 2 and 3 containing 10% and 15% cassava leaf flour were 11.35% and 12.03%, respectively.

Extrusion process

The extrusion process was performed using a co-rotating twin screw extruder (model BC-21, No 124, Cleextral, Firminy, France). According to the design, the barrel temperature at zone three of the extrusion was varied between 80 to 120°C (Table 1). Adjustments of flour feed rate and water flow rate based on the required process parameters were done after a pre-trial experiment. Flour feed rate and screw speed were set at 34.62 g/min and 400 rpm, respectively, following a pre-trial experiment. After the extrusion variables reached at steady state as designed, the extruded samples were collected and dried using an oven at 60°C for 4 hours. Then the samples were milled (high speed multi-function comminutor) into flour using 100-µm mesh size and sealed in polyethylene bags and stored in a desiccator until nutritional analyses were conducted.

Determination of functional properties

Water absorption and water solubility indices were determined according to Yagci and Gogus (2008). Exactly 0.5 g sample was dispersed into plastic centrifuge tube containing 10 ml distilled water. After standing for 30 min with intermittent shaking every 5 min, the sample was centrifuged at 1800 × g for 15 minutes. The supernatant was decanted into a tarred aluminium pan and dried at 105°C until constant weight. The weight of the gel remaining in the centrifuge tube was weighed. Equations 1 and 2 were used to calculate the water absorption and solubility indices, respectively.

Table 1. Process variables with their levels used in the Box Behnken design.

| Independent variables | Level of coded variables | | |
|-------------------------|--------------------------|--------|--------|
| | Low | Medium | High |
| Barrel temperature (°C) | -1(80) | 0(100) | 1(120) |
| Feed composition (%) | -1(5) | 0(10) | 1(15) |
| Feed moisture (%) | -1(14) | 0(16) | 1(18) |

$$WAI(g/g) = \frac{\text{Weight gain of gel}}{\text{Dry weight of sample}} \quad (\text{Eq. 1})$$

$$WSI(\%) = \frac{\text{Weight of dry solids in supernatant}}{\text{Dry weight of sample}} \times 100 \quad (\text{Eq. 2})$$

Determination of *in vitro* protein digestibility

The *in vitro* protein digestibility of cassava leaf flour was determined according to Galal et al. (2013). Exactly 200 mg cassava leaf flour was placed in a 50 ml centrifuge tube and incubated with 1.5 mg of pepsin in 15 ml of 0.1 N HCL at 37°C for 3 hours and neutralized with 7.5 ml of 0.2 M NaOH. This was followed by addition of 4 mg pancreatin in 7.5 ml phosphate buffer and incubated at 37°C for 24 hours. Then, 5 mg of Tri-chloro acetic acid (TCA) was added to stop the reaction and centrifuged at 5000 × g for 10 minutes. The mixture was then filtered through Whatman No. 4 filter paper. The supernatant was dried at 50°C and followed by assaying for nitrogen using micro-Kjeldahl method according to Galal et al. (2013) and the *in vitro* protein digestibility was calculated using equation 3.

$$\text{In vitro protein digestibility} = \frac{x-y}{x} \times 100 \quad (\text{Eq. 3})$$

Where; X is percentage of protein in the sample before digestion and Y is percentage of protein in the sample after enzymatic digestion.

Mineral analyses

Minerals (Ca, K, Mg and Fe) content of the fermented cassava leaf flour samples were analysed using atomic absorption spectrophotometer according to AOAC (2005). Each minerals was analyzed using their respective wavelengths as follows; Ca (422.7 nm), K (766.5 nm), Mg (285.2 nm) and Fe (371.9) according to Ozbek and Akman (2016).

Fitted model validation

Coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), predicted coefficient of determination

(R^2), lack of fit and coefficient of variation (C.V) were used to check if the fitted models provide an adequate estimation to the real system. The coefficient of determination (R^2) value close to unity and R^2_{adj} close to R^2 ensure satisfactory fitting of the model to the real system. Probability value (P) of each responses was also used to check for the significance of each factor and interaction between the factors. The smaller the p-value, the more significant is the corresponding coefficients (Dhawane et al., 2015).

Optimization of extrusion variables and responses

Numerical optimization of the extrusion variables and responses were done using Design-Expert to identify the best combination of independent variables which could produce optimum responses.

Experimental design and statistical analysis

Response surface methodology with Box-Behnken design was used. Preliminary trials were conducted to select the number and range of process variables (Table 1). A second order polynomial regression equation was modeled on the basis of the experimental data and optimum parameters defined using Design-Expert (DX13, Stat Ease Inc. Minneapolis, MN, USA) software and the following empirical model present the relationships among process and response variables.

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_{11} + \beta_{22}X_{22} + \beta_{33}X_{33} + \beta_{12}X_{12} + \beta_{13}X_{13} + \beta_{23}X_{23} + \varepsilon \quad (\text{Eq. 4})$$

Where; Y = estimated response, β_0 , β_i , β_{ii} and β_{ij} are coefficients of the constant term, the linear terms, the quadratic terms and the interaction terms, respectively; X_1 = extrusion temperature, X_2 = feed composition and X_3 = feed moisture and ε = the random error.

The response surface methodology procedure of Design-Expert (DX13, Stat Ease Inc. Minneapolis, MN, USA) software was used to analyse the experimental data based on the Box-Behnken design matrix (Table 2). The significance and validity of regression model equation were analysed using analysis of variance (ANOVA).

Table 2. Outline of Box Behenken experimental design matrix.

| Experimental runs | Independent variables | | |
|-------------------|-----------------------|--------------------|--------------------|
| | X ₁ (°C) | X ₂ (%) | X ₃ (%) |
| 1 | +1(120) | 0(10) | +1(18) |
| 2 | 0(100) | +1(15) | -1(14) |
| 3 | 0(100) | 0(10) | 0(16) |
| 4 | -1(80) | +1(15) | 0(16) |
| 5 | 0(100) | 0(10) | 0(16) |
| 6 | 0(100) | -1(5) | +1(18) |
| 7 | +1(120) | -1(5) | 0(16) |
| 8 | -1(80) | 0(10) | -1(14) |
| 9 | 0(100) | +1(15) | +1(18) |
| 10 | +1(120) | +1(15) | 0(16) |
| 11 | -1(80) | 0(10) | +1(18) |
| 12 | +1(120) | 0(10) | -1(14) |
| 13 | 0(100) | 0(10) | 0(16) |
| 14 | 0(100) | -1(5) | -1(14) |
| 15 | -1(80) | -1(5) | 0(16) |

Key: X₁ = Extrusion temperature, X₂ = Feed composition, X₃ = Feed moisture.

RESULTS

Functional properties of maize-cassava leaf composite instant flour

Effect of extrusion cooking variables on water absorption index (WAI) and water solubility index (WSI) of the resultant instant porridge flour is shown in Table 3. Water absorption index ranged from 4.7 to 7.3 g/g, whereas water solubility index ranged from 9.43 to 22.75%. A combination of feed composition, feed moisture and extrusion temperature had a significant effect on water absorption and water solubility indices of the resultant instant porridge flour as shown in Table 4. Instant flour from a combination of 15% cassava leaf flour, 16% moisture content and extrusion temperature of 120°C had significantly highest WAI and WSI at 7.30 g and 22.75%, respectively. Instant flour with 10% cassava leaf flour, 18% moisture and extruded at 80°C had significantly lowest WAI (4.70), whereas the control sample had significantly lowest WSI (9.43%). The quadratic model equation for WAI is:

$$\text{WAI (g/g)} = 6.17 + 0.6362X_1 + 0.3125X_2 - 0.2687X_3 + 0.1913X_1^2 - 0.1312X_2^2 - 0.5888X_3^2 - 0.0200X_1X_2 + 0.2975X_1X_3 - 0.0500X_2X_3 \quad (\text{Eq. 5})$$

It was observed from the equation (5) that the coefficients of extrusion temperature and feed composition are positive, while that of feed moisture is negative. Therefore, water absorption index increases significantly ($p < 0.05$) when extrusion temperature and feed composition increases, whereas water absorption index decreases significantly ($p < 0.05$) when feed moisture increases. The quadratic model equation for WSI is:

$$\text{WSI (\%)} = 16.94 + 1.75 X_1 + 3.11X_2 - 0.6750X_3 + 0.4575X_1^2 - 0.8325X_2^2 - 0.3325X_3^2 + 1.21X_1X_2 + 0.5850X_1X_3 + 1.10X_2X_3 \quad (\text{Eq. 6})$$

The quadratic equation (6) shows that, water solubility index increased significantly ($p < 0.05$) with an increase in extrusion temperature and feed composition. However, it was decreased with an increase in feed moisture content though it was not significant at $p < 0.05$.

The response surface plot (Figure 1) shows an interactive effect of extrusion temperature and feed composition on water absorption index. Accordingly, an increase in extrusion temperature and feed composition at a constant feed moisture of 16% resulted in increased water absorption index. Figure 2a shows that increase in extrusion temperature and feed composition at a constant feed moisture resulted in increased water solubility index. It was also observed (Figure 2b) that an increase in feed composition and feed moisture at a constant extrusion temperature of 100°C resulted in increased in water solubility index.

In vitro protein digestibility of maize-cassava leaf composite instant flour

The effect of extrusion cooking variables on *in vitro* protein digestibility (IVPD) of maize-cassava leaf flour composite extruded instant porridge flour is shown in Table 5. The IVPD of the extruded flour ranged from 80.54 to 89.95%. Instant flour made at 100°C extrusion temperature, 15% feed composition and 18% feed moisture had significantly ($p < 0.05$) the highest IVPD (89.95%), whereas the control

Table 3. Effect of extrusion cooking variables on functional properties of maize-cassava leaf composites extruded flour.

| Runs | Independent variables | | | Response variables | |
|---------|-----------------------|--------------------|--------------------|-----------------------------|---------------------------|
| | X ₁ (°C) | X ₂ (%) | X ₃ (%) | WAI (g/g) | WSI (%) |
| 1 | +1(120) | 0(10) | +1(18) | 6.37 ± 0.15 ^{bc} | 19.15 ± 0.07 ^b |
| 2 | 0(100) | +1(15) | -1(14) | 6.07 ± 0.03 ^{cdef} | 18.26 ± 0.04 ^c |
| 3 | 0(100) | 0(10) | 0(16) | 6.4 ± 0.10 ^{bc} | 17.53 ± 0.07 ^d |
| 4 | -1(80) | +1(15) | 0(16) | 5.87 ± 0.16 ^{ef} | 17.36 ± 0.04 ^d |
| 5 | 0(100) | 0(10) | 0(16) | 6.18 ± 0.12 ^{cde} | 15.96 ± 0.09 ^e |
| 6 | 0(100) | -1(5) | +1(18) | 4.93 ± 0.08 ^{ij} | 11.1 ± 0.17 ^j |
| 7 | +1(120) | -1(5) | 0(16) | 6.63 ± 0.15 ^b | 13.36 ± 0.06 ^h |
| 8 | -1(80) | 0(10) | -1(14) | 5.77 ± 0.15 ^{fg} | 16.15 ± 0.05 ^e |
| 9 | 0(100) | +1(15) | +1(18) | 5.37 ± 0.03 ^h | 18.76 ± 0.24 ^b |
| 10 | +1(120) | +1(15) | 0(16) | 7.3 ± 0.10 ^a | 22.75 ± 0.27 ^a |
| 11 | -1(80) | 0(10) | +1(18) | 4.7 ± 0.10 ⁱ | 13.97 ± 0.09 ^g |
| 12 | +1(120) | 0(10) | -1(14) | 6.25 ± 0.11 ^{cd} | 18.99 ± 0.10 ^b |
| 13 | 0(100) | 0(10) | 0(16) | 5.93 ± 0.10 ^{def} | 17.33 ± 0.12 ^d |
| 14 | 0(100) | -1(5) | -1(14) | 5.43 ± 0.08 ^{gh} | 14.98 ± 0.12 ^f |
| 15 | -1(80) | -1(5) | 0(16) | 5.12 ± 0.04 ^{hi} | 12.79 ± 0.05 ⁱ |
| Control | 100 | 0 | 16 | 7.06 ± 0.17 ^a | 9.43 ± 0.43 ^k |

Key: X₁ = Extrusion temperature, X₂ = Feed composition, X₃ = Feed moisture, WAI = Water absorption index, WSI = Water solubility index.

Table 4. ANOVA for water absorption and water solubility indices.

| Source | WAI (g/g) | WSI (%) |
|-------------------------------|---------------|---------------|
| Model | 0.0030 | 0.0021 |
| Linear terms | | |
| X ₁ | 0.0003 | 0.0018 |
| X ₂ | 0.0077 | 0.0001 |
| X ₃ | 0.0140 | 0.0674 |
| Quadratic terms | | |
| X ₁ ² | 0.1336 | 0.3327 |
| X ₂ ² | 0.2741 | 0.1086 |
| X ₃ ² | 0.0027 | 0.4712 |
| Interaction terms | | |
| X ₁ X ₂ | 0.8532 | 0.0323 |
| X ₁ X ₃ | 0.0339 | 0.2130 |
| X ₂ X ₃ | 0.6469 | 0.0443 |
| R ² | 0.9685 | 0.9731 |
| Adjusted R ² | 0.9119 | 0.9246 |
| Predicted R ² | 0.7234 | 0.7290 |
| Adequate precision | 15.4386 | 17.5302 |
| C.V (%) | 3.49 | 4.95 |
| Lack of fit | 0.6721 | 0.5746 |

Key: X₁ = Extrusion temperature, X₂ = Feed composition, X₃ = Feed moisture. All values represent p value except for coefficient of determinations (R², R²_{adj}, Pred. R²) and Adequate precision, P values in bold represent the significant terms at p < 0.05.

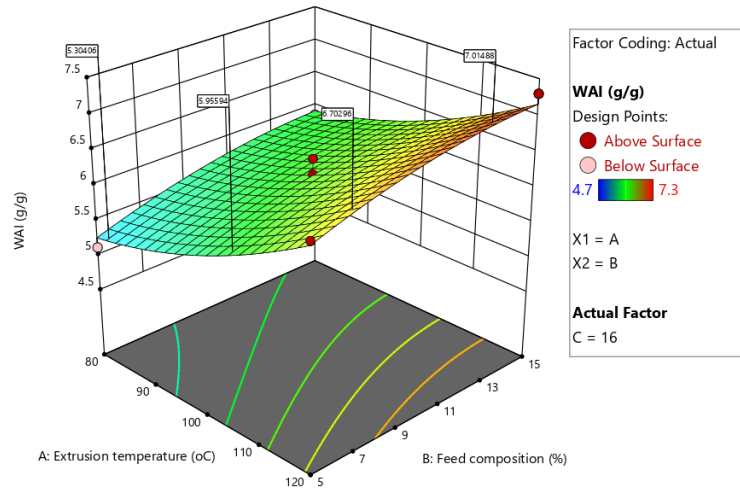
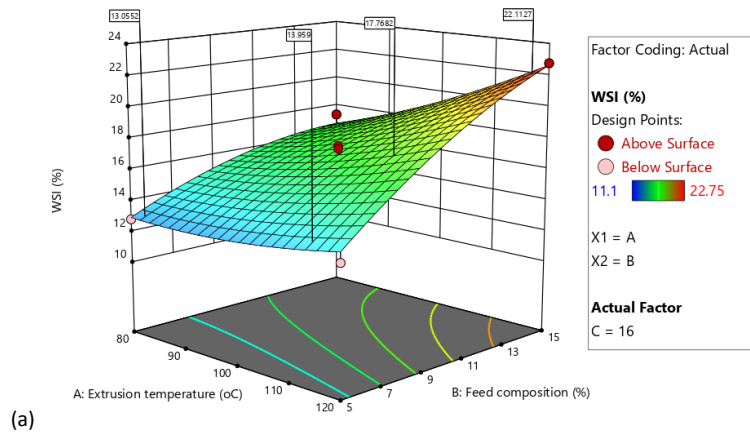
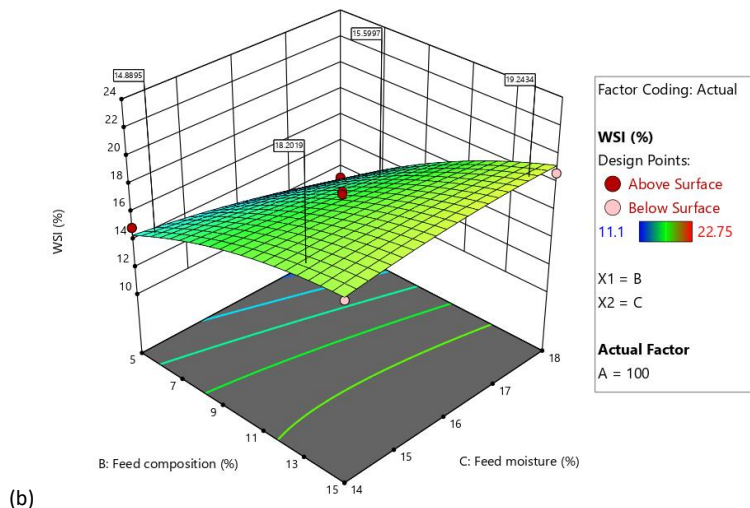


Figure 1. The 3D surface plot for effect of extrusion temperature and feed composition on water absorption index of extruded flour at constant feed moisture of 16%.



(a)



(b)

Figure 1 The 3D surface plots for effect of extrusion variables on water solubility index of extruded flour (a) feed moisture held constant at 16% (b) extrusion temperature held constant at 100°C.

Table 5. Effect of extrusion cooking variables on *in vitro* protein digestibility of maize-cassava leaf composites extruded flour.

| Runs | Independent variables | | | Response variables |
|---------|-----------------------|--------------------|--------------------|------------------------------|
| | X ₁ (°C) | X ₂ (%) | X ₃ (%) | IVPD (%) |
| 1 | +1(120) | 0(10) | +1(18) | 87.57 ± 2.81 ^{abcd} |
| 2 | 0(100) | +1(15) | -1(14) | 89.1 ± 1.21 ^{abc} |
| 3 | 0(100) | 0(10) | 0(16) | 87.44 ± 2.53 ^{abcd} |
| 4 | -1(80) | +1(15) | 0(16) | 89.17 ± 0.22 ^{ab} |
| 5 | 0(100) | 0(10) | 0(16) | 87.93 ± 0.39 ^{abcd} |
| 6 | 0(100) | -1(5) | +1(18) | 85.14 ± 1.64 ^{cde} |
| 7 | +1(120) | -1(5) | 0(16) | 85.35 ± 0.82 ^{bcd} |
| 8 | -1(80) | 0(10) | -1(14) | 84.8 ± 0.43 ^{cde} |
| 9 | 0(100) | +1(15) | +1(18) | 89.95 ± 0.98 ^a |
| 10 | +1(120) | +1(15) | 0(16) | 87.47 ± 0.18 ^{abcd} |
| 11 | -1(80) | 0(10) | +1(18) | 86.92 ± 0.93 ^{abcd} |
| 12 | +1(120) | 0(10) | -1(14) | 88.83 ± 1.04 ^{abc} |
| 13 | 0(100) | 0(10) | 0(16) | 85.97 ± 0.36 ^{abcd} |
| 14 | 0(100) | -1(5) | -1(14) | 83.97 ± 0.84 ^{def} |
| 15 | -1(80) | -1(5) | 0(16) | 81.19 ± 0.86 ^{ef} |
| Control | 100 | 0 | 16 | 80.54 ± 2.00 ^f |

Key: X₁ = Extrusion temperature, X₂ = Feed composition, X₃ = Feed moisture, IVPD = *in vitro* protein digestibility

Table 6. ANOVA for *in vitro* protein digestibility (IVPD).

| Source | IVPD (%) |
|-------------------------------|---------------|
| Model | 0.0051 |
| Linear terms | |
| X ₁ | 0.0221 |
| X ₂ | 0.0003 |
| X ₃ | 0.2084 |
| Quadratic terms | |
| X ₁ ² | 0.1426 |
| X ₂ ² | 0.1829 |
| X ₃ ² | 0.1864 |
| Interaction terms | |
| X ₁ X ₂ | 0.0127 |
| X ₁ X ₃ | 0.0800 |
| X ₂ X ₃ | 0.7179 |
| R ² | 0.9608 |
| Adjusted R ² | 0.8901 |
| Predicted R ² | 0.7495 |
| Adequate precision | 14.0641 |
| C.V (%) | 0.8894 |
| Lack of fit | 0.8351 |

Key: X₁ = Extrusion temperature, X₂ = Feed composition, X₃ = Feed moisture. All values represent p value except for coefficient of determinations (R², R² adj, Pred. R²) and Adequate precision, P values in bold represent the significant terms at p < 0.05.

sample had the lowest IVPD (80.54%). The ANOVA (Table 6) shows that IVPD was significantly (p<0.05) different by the linear and interaction terms of extrusion temperature and feed composition. The quadratic model equation for IVPD is:

$$\text{IVPD (\%)} = 87.11 + 0.8925X_1 + 2.47X_2 + 0.3938X_3 - 0.6979X_1^2 - 0.6204X_2^2 + 0.6146X_3^2 - 1.47X_1X_2 - 0.8450X_1X_3 - 0.1475X_2X_3 \quad (\text{Eq. 7})$$

The regression model in equation (7) shows that IVPD increased significantly with an increasing linear terms of extrusion temperature and feed composition of the extruded flour. However, IVPD decreased significantly with an increasing interaction terms of extrusion temperature and feed composition.

The response surface plot (Figure 3) shows an interactive effect of extrusion temperature and feed composition on *in vitro* protein digestibility. Thus, an increase in extrusion temperature and feed composition at a constant feed moisture of 16% resulted in increased *in vitro* protein digestibility of extruded flour.

Mineral contents of maize-cassava leaf composite instant flour

The effect of extrusion cooking variables on mineral contents of extruded flour is shown in Table 7. Calcium, potassium, magnesium and iron contents of the extruded flour ranged from 81.19 to 346.25 mg/100g, 217.49

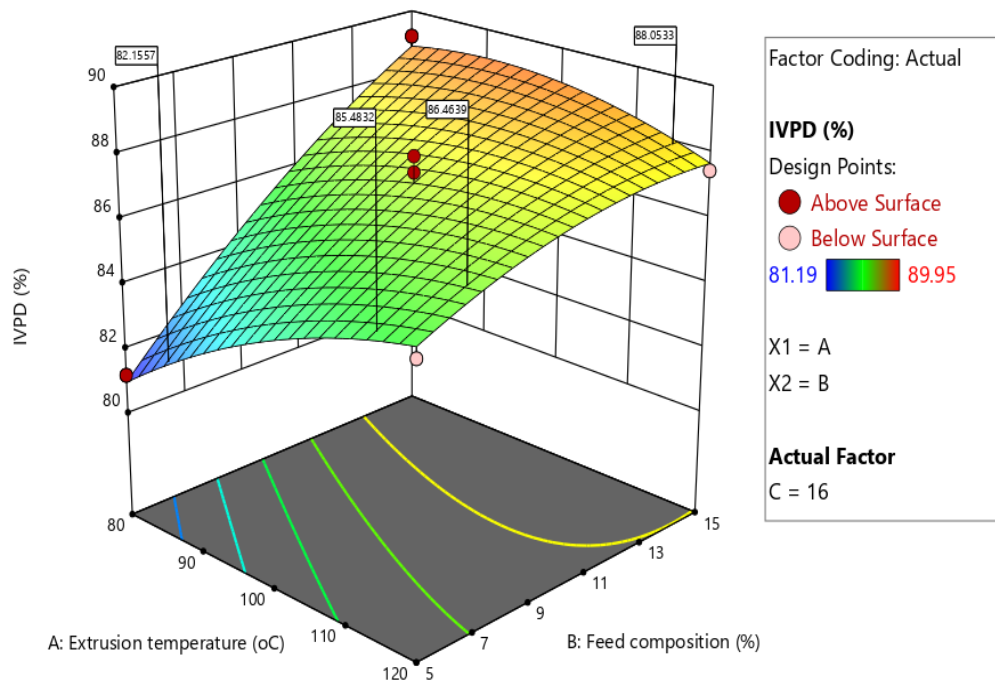


Figure 3. The 3D surface plot for effect of extrusion temperature and feed composition on *in vitro* protein digestibility of extruded flour at constant feed moisture of 16%.

Table 7. Effect of extrusion cooking variables on functional properties of maize-cassava leaf composites extruded flour.

| Runs | Independent variables | | | Response variables | | | |
|---------|-----------------------|--------------------|--------------------|-----------------------------|-----------------------------|------------------------------|--------------------------------|
| | X ₁ (°C) | X ₂ (%) | X ₃ (%) | Ca (mg/100g) | K (mg/100g) | Mg (mg/100g) | Fe (mg/100g) |
| 1 | +1(120) | 0(10) | +1(18) | 315.18 ± 4.02 ^e | 190.7 ± 0.89 ^{fg} | 117.6 ± 1.03 ^{de} | 16.37 ± 0.14 ^{bcd} |
| 2 | 0(100) | +1(15) | -1(14) | 332.3 ± 0.90 ^b | 213.18 ± 0.38 ^b | 143.59 ± 2.11 ^{ab} | 24.17 ± 0.86 ^a |
| 3 | 0(100) | 0(10) | 0(16) | 304.87 ± 0.17 ^f | 177.54 ± 0.34 ^j | 112.84 ± 1.89 ^{ef} | 14.83 ± 0.84 ^{bcddef} |
| 4 | -1(80) | +1(15) | 0(16) | 346.25 ± 0.94 ^a | 217.49 ± 0.99 ^a | 148.7 ± 5.75 ^a | 26.5 ± 3.29 ^a |
| 5 | 0(100) | 0(10) | 0(16) | 306.74 ± 2.01 ^f | 187.46 ± 1.13 ^{gh} | 111.28 ± 0.71 ^{efg} | 11.59 ± 0.15 ^{efg} |
| 6 | 0(100) | -1(5) | +1(18) | 286.83 ± 0.92 ^h | 181.5 ± 0.33 ^j | 97.85 ± 0.77 ^{hi} | 10.87 ± 1.11 ^{fg} |
| 7 | +1(120) | -1(5) | 0(16) | 294.63 ± 3.26 ^g | 193.95 ± 2.06 ^{ef} | 95.44 ± 1.12 ⁱ | 10.96 ± 0.05 ^{fg} |
| 8 | -1(80) | 0(10) | -1(14) | 325.77 ± 4.02 ^{cd} | 205.8 ± 0.35 ^d | 134.19 ± 1.17 ^c | 17.44 ± 0.14 ^{bc} |
| 9 | 0(100) | +1(15) | +1(18) | 325.59 ± 1.00 ^{cd} | 210.26 ± 0.37 ^{bc} | 140.95 ± 0.99 ^{bc} | 25.32 ± 0.82 ^a |
| 10 | +1(120) | +1(15) | 0(16) | 324.38 ± 0.93 ^{cd} | 186.61 ± 1.23 ^h | 137.4 ± 0.85 ^{bc} | 23.41 ± 1.01 ^a |
| 11 | -1(80) | 0(10) | +1(18) | 321.8 ± 0.61 ^d | 196.78 ± 0.27 ^e | 121.62 ± 1.03 ^d | 18.74 ± 1.06 ^b |
| 12 | +1(120) | 0(10) | -1(14) | 329.03 ± 0.23 ^{bc} | 208.86 ± 1.12 ^{cd} | 116.05 ± 2.97 ^{def} | 15.62 ± 0.91 ^{bcdde} |
| 13 | 0(100) | 0(10) | 0(16) | 302.34 ± 0.22 ^f | 186.89 ± 0.49 ^h | 104.98 ± 5.95 ^{gh} | 13.78 ± 0.17 ^{cdef} |
| 14 | 0(100) | -1(5) | -1(14) | 295.59 ± 3.69 ^g | 195.47 ± 2.31 ^e | 109.28 ± 0.30 ^{fg} | 12.75 ± 0.90 ^{def} |
| 15 | -1(80) | -1(5) | 0(16) | 290.09 ± 0.24 ^{gh} | 170.21 ± 1.11 ^k | 111.06 ± 0.02 ^{efg} | 14.22 ± 3.32 ^{cdef} |
| Control | 100 | 0 | 16 | 81.19 ± 2.66 ⁱ | 47.46 ± 2.58 ^l | 79.10 ± 1.35 ^j | 7.67 ± 0.93 ^g |

Key: X₁ = Extrusion temperature, X₂ = Feed composition, X₃ = Feed moisture.

mg/100g, 79.10 to 148.7 mg/100g and 7.67 to 26.5 mg/100g, respectively. Instant flour prepared at extrusion temperature of 80°C, feed composition of 15% and feed moisture of 16% had significantly ($p < 0.05$) the highest mineral contents whereas the lowest values were

observed for the control sample. The ANOVA (Table 8) shows that calcium and potassium contents were significantly ($p < 0.05$) different by the linear terms of feed composition and feed moisture, quadratic terms of extrusion temperature and feed moisture, interaction terms

Table 8. ANOVA for mineral contents of the extruded flour.

| Source | Ca (mg/100g) | K (mg/100g) | Mg (mg/100g) | Fe (mg/100g) |
|-------------------------------|-------------------|---------------|-------------------|-------------------|
| Model | 0.0002 | 0.0028 | 0.0002 | 0.0008 |
| Linear terms | | | | |
| X ₁ | 0.0656 | 0.4176 | 0.0016 | 0.0265 |
| X ₂ | <0.0001 | 0.0007 | <0.0001 | <0.0001 |
| X ₃ | 0.0129 | 0.0122 | 0.0249 | 0.7129 |
| Quadratic terms | | | | |
| X ₁ ² | 0.0010 | 0.0999 | 0.0069 | 0.0211 |
| X ₂ ² | 0.3076 | 0.1298 | 0.0049 | 0.0032 |
| X ₃ ² | 0.0065 | 0.0021 | 0.0080 | 0.0529 |
| Interaction terms | | | | |
| X ₁ X ₂ | 0.0082 | 0.0011 | 0.4755 | 0.9462 |
| X ₁ X ₃ | 0.1734 | 0.3123 | 0.0531 | 0.8275 |
| X ₂ X ₃ | 0.7553 | 0.2325 | 0.1774 | 0.2618 |
| R ² | 0.9888 | 0.9693 | 0.9902 | 0.9821 |
| Adjusted R ² | 0.9687 | 0.9140 | 0.9725 | 0.9500 |
| Predicted R ² | 0.8521 | 0.8254 | 0.9620 | 0.9012 |
| Adequate precision | 22.8041 | 14.7264 | 22.5281 | 15.6251 |
| C.V (%) | 0.7530 | 0.8221 | 1.27 | 7.00 |
| Lack of fit | 0.2861 | 0.8750 | 0.9599 | 0.8835 |

Key: X₁ = Extrusion temperature, X₂ = Feed composition, X₃ = Feed moisture. All values represent p value except for coefficient of determinations (R², R² adj, Pred. R²) and Adequate precision; P values in bold represent the significant terms at p<0.05.

of extrusion temperature and feed composition. Magnesium content was significantly (p<0.05) different by all linear terms and quadratic terms. However, iron content was significantly (p<0.05) different by linear and quadratic terms of extrusion temperature and feed composition. The quadratic model equation for mineral contents are:

$$\text{Ca (mg/100g)} = 304.65 - 2.59X_1 + 20.17X_2 - 4.16X_3 + 11.03X_1^2 - 1.84X_2^2 + 7.27X_3^2 - 6.60X_1X_2 - 2.47X_1X_3 + 0.5125X_2X_3 \quad (\text{Eq. 8})$$

The quadratic model equation (8) shows that calcium content increased significantly (p<0.05) with increase in feed composition. However, calcium content decreases significantly (p<0.05) with increase in feed moisture.

$$\text{K (mg/100g)} = 183.96 - 1.27X_1 + 10.80X_2 - 5.51X_3 + 4.27X_1^2 + 3.83X_2^2 + 12.30X_3^2 - 13.65X_1X_2 - 2.29X_1X_3 + 2.76X_2X_3 \quad (\text{Eq. 9})$$

The quadratic model equation (9) shows that potassium content increased significantly (p<0.05) with increase in feed composition. However, the potassium content decreases significantly (p<0.05) with increase in feed moisture.

$$\text{Mg (mg/100g)} = 109.70 - 6.14X_1 + 19.63X_2 - 3.14X_3 + 6.45X_1^2 - 7.00X_2^2 + 6.22X_3^2 + 1.08X_1X_2 + 3.53X_1X_3 + 2.20X_2X_3 \quad (\text{Eq. 10})$$

The quadratic model equation (10) shows that magnesium content increased significantly (p<0.05) with increase in feed composition. However, magnesium content decreases significantly (p<0.05) with increase in extrusion temperature and feed moisture.

$$\text{Fe (mg/100g)} = 13.40 - 1.32X_1 + 6.32X_2 + 0.1650X_3 + 2.07X_1^2 + 3.30X_2^2 + 1.57X_3^2 + 0.0425X_1X_2 - 0.1375X_1X_3 + 0.7575X_2X_3 \quad (\text{Eq. 11})$$

The quadratic model equation (11) shows that iron content increased significantly (p<0.05) with increase in feed composition. However, iron content decreased significantly (p<0.05) with increase in extrusion temperature.

The response surface plot (Figure 4) shows an interactive effect of extrusion temperature and feed composition on calcium content of extruded flour. Accordingly, an increase in extrusion temperature and feed composition at a constant feed moisture of 16% resulted in increased

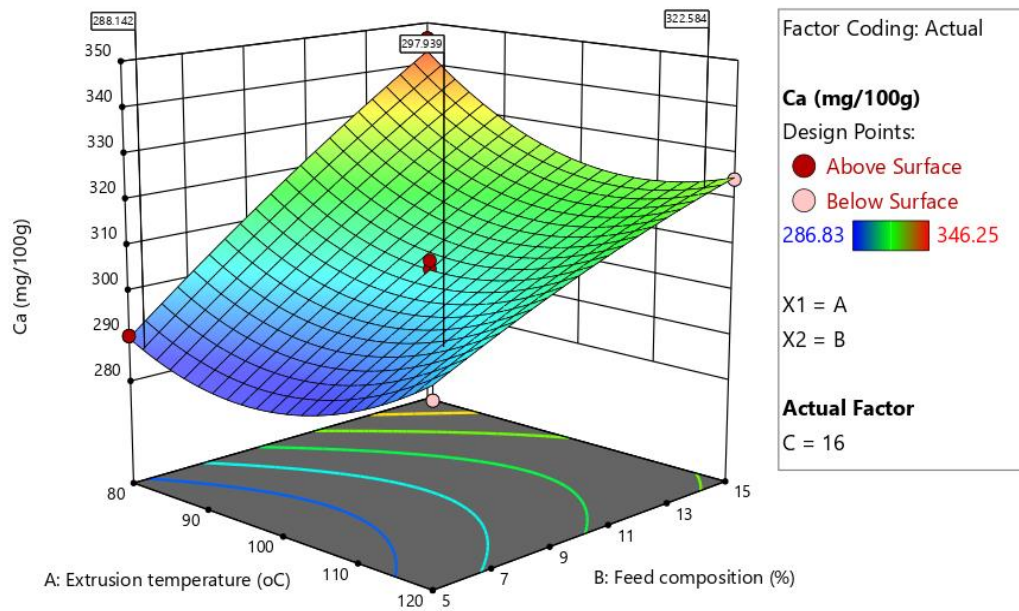


Figure 4. The 3D surface plot for effect of extrusion temperature and feed composition on Calcium content of extruded flour at constant feed moisture of 16%.

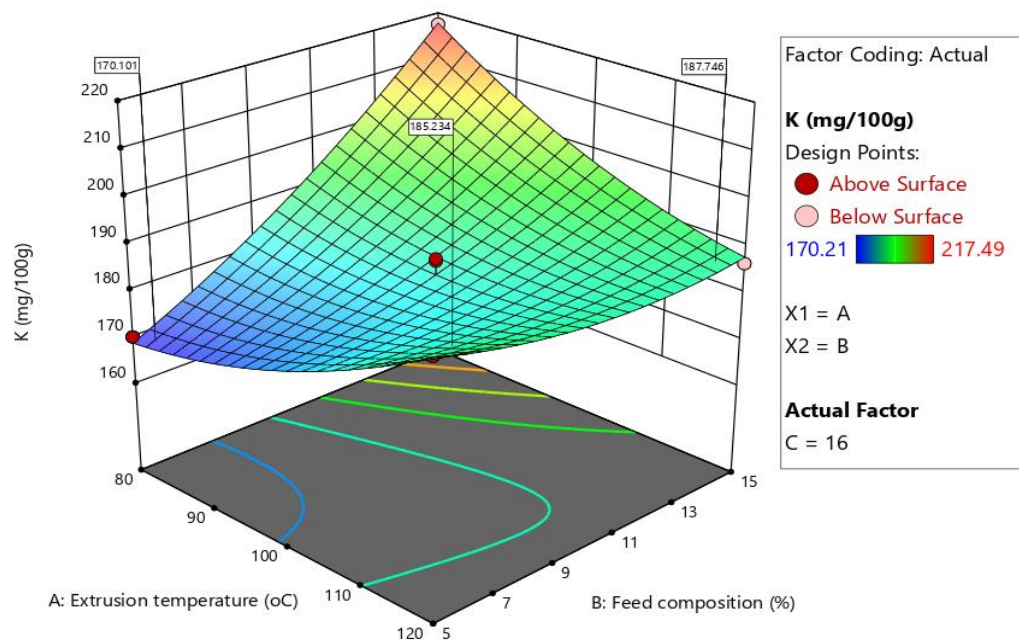


Figure 5. The 3D surface plot for effect of extrusion temperature and feed composition on Potassium content of extruded flour at constant feed moisture 16%.

calcium content. Response surface plot (Figure 5) shows that an increase in extrusion temperature and feed composition at a constant feed moisture of 16% resulted in increased potassium content.

Correlation between responses

The correlation between functional properties, *in vitro* protein digestibility and minerals are shown in Table 9.

Table 9. Correlation (Pearson correlation coefficients) matrix between responses.

| | WAI | WSI | Ca | K | Mg | Fe | IVPD |
|------|----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------|
| WAI | 1.00000 | | | | | | |
| WSI | 0.32329 (0.0250) | 1.00000 | | | | | |
| Ca | -0.32412 (0.0246) | 0.65842 (<.0001) | 1.00000 | | | | |
| K | -0.36877 (0.0099) | 0.59095 (<.0001) | 0.97893 (<.0001) | 1.00000 | | | |
| Mg | -0.12239 (0.4073) | 0.70896 (<.0001) | 0.71763 (<.0001) | 0.69276 (<.0001) | 1.00000 | | |
| Fe | -0.07029 (0.6350) | 0.67539 (<.0001) | 0.60260 (<.0001) | 0.57892 (<.0001) | 0.94352 (<.0001) | 1.00000 | |
| IVPD | 0.03582 (0.8090) | 0.69631 (<.0001) | 0.66370 (<.0001) | 0.65746 (<.0001) | 0.63981 (<.0001) | 0.62836 (<.0001) | 1.00000 |

Values in bracket represents p value.

Table 10. Optimum values for extrusion and response variables.

| Variables | Lower Limit | Upper Limit | Optimum |
|-------------------------------|-------------|-------------|---------|
| A: Extrusion temperature (oC) | 80 | 120 | 101 |
| B: Feed composition (%) | 5 | 15 | 12 |
| C: Feed moisture (%) | 14 | 18 | 16 |
| WAI (g/g) | 4.7 | 7.3 | 6.25 |
| WSI (%) | 11.1 | 22.75 | 18.3 |
| IVPD (%) | 81.19 | 89.95 | 88.23 |
| Ca (mg/100g) | 286.83 | 346.25 | 312.96 |
| K (mg/100g) | 170.21 | 217.49 | 189.14 |
| Mg (mg/100g) | 95.44 | 148.7 | 120.08 |
| Fe (mg/100g) | 10.87 | 26.5 | 12.21 |

Water solubility index had a highly significant ($p < 0.0001$) strong positive correlation between minerals and *in vitro* protein digestibility. However, water absorption index was not significant ($p > 0.05$) except showing a weak significant ($p < 0.05$) correlation with water solubility index, calcium and potassium.

Optimization of extrusion and response variables

The optimum extrusion variables and responses are shown in Table 10. The optimum extrusion temperature, feed composition and feed moisture were 101°C, 12% and 16%, respectively. Optimum values of WAI, WSI, IVPD, Ca, K, Mg and Fe were 6.245 g, 18.3%, 88.23%, 312.96 mg/100g, 189.14 mg/100g, 120.08 mg/100g and 12.21 mg/100g, respectively. Figure 6 shows a deviation of responses from the actual values as influenced by extrusion variables.

DISCUSSION

The high utilization and consumption levels of maize in developing countries calls for application of different processing techniques and supplementation with other ingredients to help introduce variety as well as improve the functionality and nutritional quality of maize-based foods (Sefa-Dedeh et al., 2004).

In this study, extrusion cooking was used to produce a blended instant flour with improved functional properties, *in vitro* protein digestibility and mineral contents. The Box Behnken design was used to optimize extrusion parameters using RSM. The RSM was found to be an effective tool in explaining the effect of extrusion process variables on functional properties, *in vitro* protein digestibility and mineral contents of maize-cassava leaf composite instant porridge flour.

Water absorption index depicts the amount of water held by the extruded flour and this is triggered by the

Factor Coding: Actual

All Responses

Actual Factors

A = 101.017

B = 12.3532

C = 16.3876

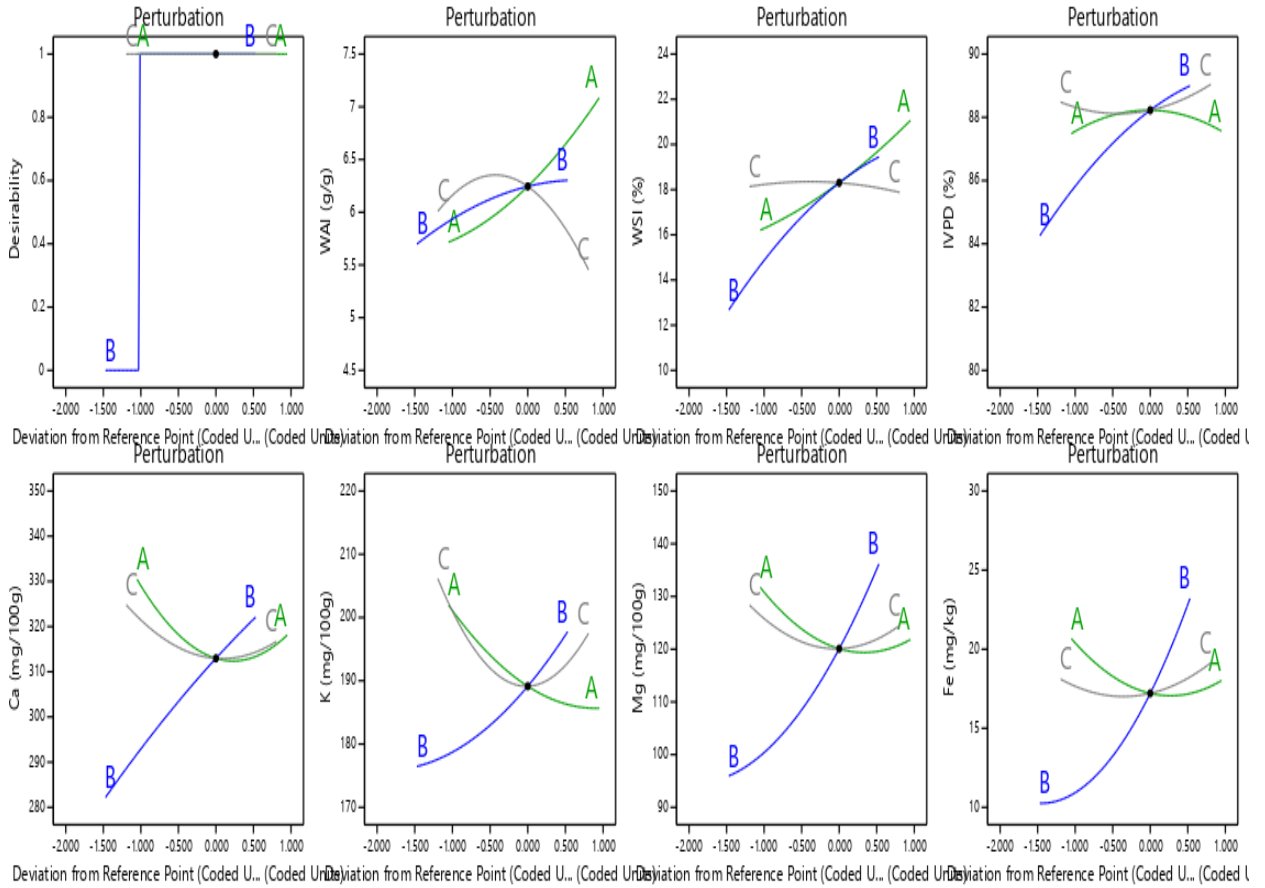


Figure 6. Perturbation plots for each response and extrusion variables.

gelatinization and melting of molecules (Pasqualone et al., 2021). It is attributed to the dispersion of starch in excess water and the dispersion is increased by the degree of starch damage due to gelatinization and extrusion induced fragmentation (Yagci and Gogus, 2008). In this study, all the linear terms, the quadratic term of feed moisture and interaction terms of extrusion temperature and feed moisture of the regression model of WAI were significant at $p < 0.05$ (Table 4). About 97% of the variation in WAI of the extruded flour could be linked to the extrusion cooking variables based on its R^2 value of 0.9685 as shown in Table 4. The adequate precision; a signal to noise ratio greater than 4 indicates goodness of fit (Banki et al., 2021), in this case an adequate signal was obtained with a value of 15.44. Also, lack of fit which serves as a tool for measuring the failure of a model is expected not to be significant, in this study a p value of 0.6721 was obtained. The quadratic model equation (4) shows that, the coefficients of extrusion temperature and feed composition

are positive, but that of feed moisture is negative. Therefore, increase in extrusion temperature and feed composition may increase water absorption index, whereas increase in feed moisture may decrease water absorption index of the extruded flour. The response surface plot (Figure 1) shows the interactive effect of extrusion temperature and feed composition at a constant feed moisture of 16% on water absorption index. Accordingly, with increasing extrusion temperature up to 120°C and feed composition up to 15%, WAI increased. This might be due to an increase in extrusion temperature that may have caused fiber structure to be more unfolded by releasing free hydroxyl groups from cellulose and increasing the ability to bind with water (Tabibloghmany et al., 2020). Moreover, the denaturation of proteins that occurs during extrusion at higher temperature increases the availability of polar groups of amino acids, which enhances hydrophobicity and consequently increases the water absorption capacity of the extruded product (Altan et

al., 2009). In the study conducted by Lazou and Krokida (2010), it was explained that protein denaturation occurring in high extrusion temperature conditions is the main phenomena influencing the hydration properties of the extrudate and increases the products characteristics to have more water affinity.

Water solubility index is often used as an index for the degradation of molecular components, indicating the amount of soluble polysaccharides released from fiber components during extrusion (Tabibloghmany et al., 2020). In this study, water solubility index ranged from 9.43 to 22.75%, which is lower than the finding of Seth et al. (2015) who reported water solubility index in the range of 18.13 to 30.39% for yam-corn-rice based extruded snack. However, the present finding is in agreement with the finding of Kothakota (2013) who reported water solubility index of 11.2 to 20.8% for broken rice flour, dehydrated pineapple waste and red gram powder composite extruded flour, but slightly higher than the finding of Grossmann et al. (1998) who reported WSI in the range of 2.94 to 16.04% for malted and extruded corn flour. In the current study, the linear terms of extrusion temperature and feed composition, and the interaction terms of extrusion temperature and feed composition as well as interaction terms of feed composition and feed moisture for WSI were significant at $p < 0.05$ (Table 4). Therefore, about 97% of the variation in WSI of the extruded flour could be linked to the extrusion cooking variables based on its R^2 value of 0.9731. Response surface plot (Figure 2a) indicates that an increase in extrusion temperature up to 120°C and feed composition up to 15% at a constant feed moisture of 16%, resulted in an increase in water solubility index. This may be due to an increase in the amount of soluble starch because of gelatinization and lateral expansion of starch occurred at higher temperature during extrusion (Yousf et al., 2017). Response surface plot (Figure 2b) also explains that an increase in feed composition and feed moisture at constant extrusion temperature resulted in increase in water solubility index of the extruded flour. This may be related to the modification of fiber coming from cassava leaf flour during extrusion causing increase in WSI. Similar behaviour was observed by Altan et al. (2009) for barley based extrudates from fruit and vegetable by-products.

Proteins are essential macro-nutrients for human diet and its digestibility defines the quality of proteins (Qi et al., 2021). The digestibility specifies the protein quantity absorbed by an organism relative to the consumed amount and depends on the protein structure, previous processing, and the presence of compounds limiting the digestion (Kamani et al., 2021). The extrusion cooking variables in this study contributed 96% for the variations in protein digestibility (Table 6). The *in vitro* protein digestibility was significantly ($p < 0.05$) improved up to 89.95% when fermented cassava leaf flour is supplemented with maize flour during extrusion. This might be due to the increase in availability of protein in cassava leaf flour resulting from the fermentation process in addition to the extrusion

effects. The increase in protein digestibility with an increase in extrusion temperature and feed composition at a constant feed moisture of 16% (Figure 3) might be due to inactivation and/or reduction of anti-nutrients which limits the availability of nutrients (Omosebi et al., 2018; Batista et al., 2010). In addition, denaturation of proteins during extrusion process can expose sites that are susceptible to the enzymatic hydrolysis (Bekele et al., 2021). A similar trend was observed by Ukeyima et al. (2021) who found an improvement of *in vitro* protein digestibility to 92.91% for maize flour, peanut flour and beet roots flour composite extruded flour.

Calcium content was significantly ($p < 0.05$) increased from 81.19 to 346.25 mg/100 g when cassava leaf flour proportion in the composite increases (Table 7). This might be attributed to the higher content of calcium in cassava leaf flour, which can contribute to calcium increase in the composite flour. Cassava leaves are a good source of calcium (0.43 to 1.14 g/100g dry weight) depending on the maturity level, the mature the leaves, the higher calcium content (Ravindran and Ravindran, 1988). However, calcium content of the extruded flour was not affected significantly ($p > 0.05$) by extrusion temperature. Extrusion temperature is not supposed to cause a significant change in mineral contents of extruded products as they are stable at higher temperature. This agrees with Nahemiah et al. (2018) who stated that minerals are heat stable and unlikely to be lost during extrusion cooking. The extrusion cooking variables contributed about 99% variations in calcium content of which the maximum contribution was by linear terms of feed composition followed by quadratic terms of feed moisture, interaction terms of extrusion temperature and feed composition, and linear terms of feed moisture (Table 8). The response surface plot (Figure 4) also explains that the change in calcium content was much attributed to feed composition. Calcium content of the extruded flour had a highly significant ($p < 0.0001$) positive correlation with water solubility index, potassium, magnesium, iron and *in vitro* protein digestibility.

Potassium content significantly ($p < 0.05$) increased from 47.46 to 217.49 mg/100g when cassava leaf proportion increased up to 15% in the composite. The increase in potassium content might be due to the contribution of the cassava leaf flour in the composite. Cassava leaves contain 1.38 g/100g to 2.26 g/100g of potassium depending on the level of maturity, the mature the leaves, the lower amount of potassium (Ravindran and Ravindran, 1988). About 97% of variations in potassium content of the extruded flour caused by the extrusion cooking variables (Table 8). The maximum variation was due to linear terms of feed composition followed by interaction terms of extrusion temperature and feed moisture, quadratic terms of feed moisture, and linear terms of feed moisture. It was also observed in Figure 5 that an increase in extrusion temperature and feed composition at a constant feed moisture of 16% resulted in increased potassium content.

Magnesium content was significantly ($p < 0.05$) increased

from 79.10 to 148.7 mg/100g when cassava leaf proportion increases up to 15% in the composite. This could be attributed to the higher amount of magnesium (0.26 g/100g to 0.37 g/100g) in cassava leaves (Ravindran and Ravindran, 1988). A significant ($p < 0.05$) effect on magnesium content was observed by both linear and quadratic terms of extrusion temperature, feed composition and feed moisture. The quadratic model equation (Eq. 10) shows that magnesium content was negatively affected by linear terms of extrusion temperature and feed moisture. The extrusion cooking variables contributed about 97% variations in magnesium content of which the maximum contribution was by linear term of feed composition followed by linear term of extrusion temperature, quadratic terms of feed composition, extrusion temperature, feed moisture, and linear term of feed moisture (Table 8). The variation in magnesium content during extrusion might be due to the reduction of anti-nutrients favours releasing of the bound minerals (Adebowale et al., 2017).

Iron is vital for erythropoiesis to thrive; it is an integral part of red blood cells (RBCs) which transports oxygen from the lungs to different organs of the body and it is a key constituent of haemoglobin (Suri et al., 2020). Iron content was significantly ($p < 0.05$) increased from 7.67 to 26.5 mg/100g when cassava leaf proportion increases up to 15% in the composite. Cassava leaves contain a significant amount of iron in the ranges between 15.2 mg/100g to 26.6 mg/100g depending on maturity, the mature the leaves the higher iron contents (Ravindran and Ravindran, 1988). It can be observed from the regression model (Table 8), that the linear effect of extrusion temperature and feed composition, as well as the quadratic effect of extrusion temperature and feed composition on iron content, were found to be significant ($p < 0.05$). The quadratic model equation (Eq. 11) shows that iron content was negatively affected by the linear terms of extrusion temperature and interaction terms of extrusion temperature and feed moisture.

Conclusion

This study has evaluated the functional properties, *in vitro* protein digestibility and some mineral contents for the extruded instant porridge flour. Maize and cassava leaf flour can be combined to produce good quality instant porridge flour. The results of this study showed that up to 15% cassava leaf flour could be incorporated into maize flour for production of instant porridge flour with improved functional properties, *in vitro* protein digestibility and mineral contents. The response surface methodology clearly optimized the response variables. The optimum extrusion variables that could give optimum functional properties, *in vitro* protein digestibility and mineral contents were; extrusion temperature (101°C), feed composition (12%) and feed moisture (16%). The bio-availability of minerals, which is crucial for assessing the mineral quality

should be explored further.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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