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***Research article***

# Effects of Bambara groundnut and butternut blend on proximate, mineral, beta-carotene and folic acid contents of sorghum flour

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**Abstract:** The refined sorghum flour (SF) used is limited in fiber and micronutrients because of bran removal during milling, and protein digestibility is poor due to kafrin crosslinking. In this research, the effects of Bambara groundnut (BG) (15%, 25%, 35%) and butternut (BU) powder (23%) blending on SF were investigated, using 100% SF as a control. The proximate, mineral, beta-carotene and folic acid compositions of the flour mix were determined. As the BG levels increased, the protein, fat, fiber, and ash contents increased significantly ($p < 0.05$), ranging between 8.62–14.19%, 2.36–3.38%, 1.37–3.04% and 0.87–2.19%, respectively. The iron, zinc, calcium and phosphorus contents in mg/100 g were 3.43–5.08, 2.96–3.74, 80.00–106.67 and 150.63–594.53, respectively. The beta-carotene (mg/100 g) and folic acid (μg/100 g) contents were <0.01–0.63 and 0.75–1.42, respectively. The mineral, beta-carotene and folic acid contents of the flour mix varied significantly ($p < 0.05$) from the control. The pro-vitamin A beta-carotene content was improved in the blend flours with the addition of BU powder, whereas, in the control sample, it was not detected (<0.01 mg/100 g). With the 35% BG blend, increases of 37% protein, 45% crude fiber, 48% iron, 26% zinc, 133% calcium and 154% folic acid contents from the control were observed. The study showed food-to-food fortification of SF with BG flour and BU powder has the potential to combat malnutrition, and the public health challenges associated with deficiencies in bioactive fibers, proteins and micronutrients (pro-vitamin A carotenoids, folic acid and minerals).

**Keywords:** Bambara groundnut; blending; butternut; nutrients; sorghum flour

## 1. Introduction

Sorghum is adapted to semi-arid and arid regions of Africa because of its drought tolerance. Sorghum foods are a staple in many African nations, including Botswana, where sorghum porridge (*Bogobe*) fermented as ting is regarded as a national staple [1]. In addition, sorghum grain is used in opaque beer (*Chibuku* or *Bojalwa Jwa Setswana*) and non-fermented porridge (*Mosokwane*) processing [2].

Sorghum grains are sources of starches, proteins, fibers, ash (minerals), lipids and B-vitamins, but they also bear anti-nutritional factors such as condensed tannins and phytic acids [3]. The presence of condensed tannins has a limiting effect on α-amylase and α-glucosidase enzymes, starch digestibility and mineral (iron and zinc) bioavailability, even though such an effect is indicated as beneficial for the reduction of hyperglycemia [4]. Phytic acid is inhibitory to mineral (iron, zinc, magnesium and calcium) bioavailability and digestive enzymes even though the intake of low phytic acid has been described as beneficial as an antioxidant, antidiabetic and, a factor for the prevention of some cancers and coronary heart disease because of its chelating ability of pro-oxidant metals [5]. The sorghum protein digestibility is known to be poor among cereal grains, and it becomes even poorer when cooked because of kafrin protein crosslinking [6], and it is also limited in the essential amino acids such as lysine and essential fatty acids like omega-3 [3]. Since the dry milling of sorghum grain leads to bran removal, sorghum flour meal ends up limited in the dietary fiber, mineral nutrients, phytic acid, B-vitamins, and lipids [7]. Bran removal also leads to the partial loss of the antioxidant-rich phenolic compounds located in the pericarp, seed coat and aleurone layer of sorghum grain [7]. Nevertheless, sorghum-based functional foods are attractive for celiac patients because it is free of allergenic wheat-type gluten proteins, for the formulation of low-calorie diets for those suffering from high glycemic load-related health complications and for its polyphenolic antioxidants [8].

The refined sorghum flour nutrients and bioactive compounds can be enhanced through food-to-food fortification. Among the underutilized crops, those with the potential to improve the sorghum flour meal for porridge quality are Bambara groundnut and butternut. Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is believed to have originated from the Sahelian region of present-day West Africa [9]. Even though the name Bambara is believed to be derived from a tribe called "Bambara", inhabitants of Mali, based on a genetic study, a recent report revealed northeastern Nigeria and northern Cameroon as the most probable origin of the crop [9,10]. The crop is currently cultivated at large scales by small-holder farmers as a drought tolerant crop on the degraded and marginal environment in the sub-Saharan region of tropical and sub-tropical Africa [11,12].

Bambara groundnut comprises 24–25% proteins, 58–62% total carbohydrates, 5.9–6.1% fat, 3.4–3.7% crude fiber, 3.6–3.8% ash [13] and 33–53% starches, with amylose in the range of 16–35% [12]. Nwadi et al. [14] reported 9.6–40.0% proteins, 3.41–6.85% fiber, 4.30–7.24% fats, 2.90–5.37% ash and 42.0–70.0% carbohydrates for the Bambara groundnut. The Bambara groundnut has a high potential to complement the essential amino acids (% protein) in cereal grains, i.e., histidine (0.59–4.09), leucine (1.33–10.22), lysine (0.99–8.54), threonine (0.61–5.22) and valine (0.71–6.47), for desirable diet formulations for human nutrition [12]. Bambara groundnut can also provide significant mineral nutrients (Mg, K, P, Fe, Mn, Cu and Zn) [9]. The high potassium and low sodium contents in the

Bambara groundnut are desirable to combat high blood pressure. The brown, red and black seed coated Bambara groundnut were reported to bear significant bioactive flavonoid compounds (rutin, myricetin and kaempferol), chlorogenic and ellagic acids [9]. After processing, if Bambara groundnut is blended with sorghum meal, there is high potential to improve the contents of essential amino acids [15] to mitigate the problem of protein-energy malnutrition, and, to some extent, the mineral nutrient deficiency challenges [14].

Butternut (*Cucurbita moschata*, Duchesne) is originated from México; its cultivation is limited in Africa (the largest producers being Algeria, Egypt, Malawi and South Africa), and because of this, it is described as an orphan crop [16]. The butternut fruit is rich in various bioactive compounds such as total carotenes 160.0–1399.4 µg/g, β-carotene 0.006–2340.000 µg/g, α-carotene 6–47 µg/g, lutein 0.03–20.6 µg/g, crude fiber 0.56–1.56%, ash 0.57–0.89%, vitamin C 22.9 mg/100 g, pectin 0.7% and total phenolic compounds 476.6 mg GAE/100 g [17]. Because of antioxidant carotenoids, phenolic compounds and pectins, the butternut fruits are purported to bear anti-diabetic, anti-hypertensive, anti-tumor, anti-cancer, immunomodulatory, antibacterial, anti-hypercholesterolemia, intestinal anti-parasitical and anti-inflammation effects [17]. The fruits are an important source for pro-vitamin A carotenoids and lutein, which are vital to prevent cataracts and age-related macular degeneration disease. Butternut fruits, unless preserved or used in various value-added products, can have high postharvest losses. The blending of butternut with sorghum meal thus has high potential to add value to the butternut, as well as improve pro-vitamin A carotenoids, phenolic antioxidants, and fiber content of the sorghum meal [16,17].

In the past, examples of food-to-food fortification to improve sorghum flour, including sorghum-cowpea composite porridges [18,19], Bambara groundnut-fortified sorghum-based kisra [20], complementary food [21] and functional and sensory properties sorghum flour porridge fortified with Bambara groundnut flour and butternut powder [22] products were reported. Bambara groundnut flour enrichment in the range 10 to 40% in different food formulations involving cereal grains and starchy tubers has been reviewed recently [14]. However, information on the blending of Bambara groundnut (BG) into refined sorghum flour, along with the addition of butternut powder to improve the protein, dietary fiber, micronutrient, pro-vitamin A carotenoid and folic acid contents does not seem to have been investigated yet. In view of this, in this work, the effects of Bambara groundnut and dried butternut powder on the nutritional properties of sorghum flour are reported.

## 2. Materials and methods

### *2.1. Food sample sources*

Sorghum flour labeled Earth Grown, Botswana Agricultural Marketing Board (BAMB), was purchased from a supermarket in Gaborone, Botswana. Bambara groundnut (brown color) was purchased from Botswana Agricultural Marketing Board (BAMB), Gaborone. Butternut variety F1 Sweetmax of yellow-orange flesh color was purchased from a supermarket in Gaborone (JGA Fourie Boerdery, Pty Ltd., produce of South Africa).

### *2.2. Sample preparation*

The Bambara groundnut was cleaned to remove the dust, broken chaff, broken grains and malformed grains, washed with distilled water and then dried in an oven cabinet drier at 60 °C for 24 h on stainless-steel trays. The dried Bambara groundnut was milled to flour by using a Cross Beater Mill (SK 300, RETSCH, Germany) fitted with a 500-µm sieve.

The butternut was washed thoroughly with tap water, the skin was peeled and removed, the remaining part was sliced with a sharp knife and then the seeds were removed. The yellow-orange flesh pulp was sliced (1.5–2.0 mm thick) in a cube and blanched in hot water (90 °C for 1 min). The blanched pieces were strained and cooled with running tap water for about 1 min without direct contact with the sample; then they were wiped with clean lint-free absorbent tissue. The edible pulp was homogenized by using a kitchen food-processing machine, dried (72 °C, 24 h) [23] in an oven on a stainless-steel tray and milled to powder by using a Cross Beater Mill (SK 300, RETSCH, Germany) fitted with a 500-µm sieve. The processing flow stages for the production of the butternut powder, Bambara groundnut flour and blending with the sorghum flour are given in Figure 1.

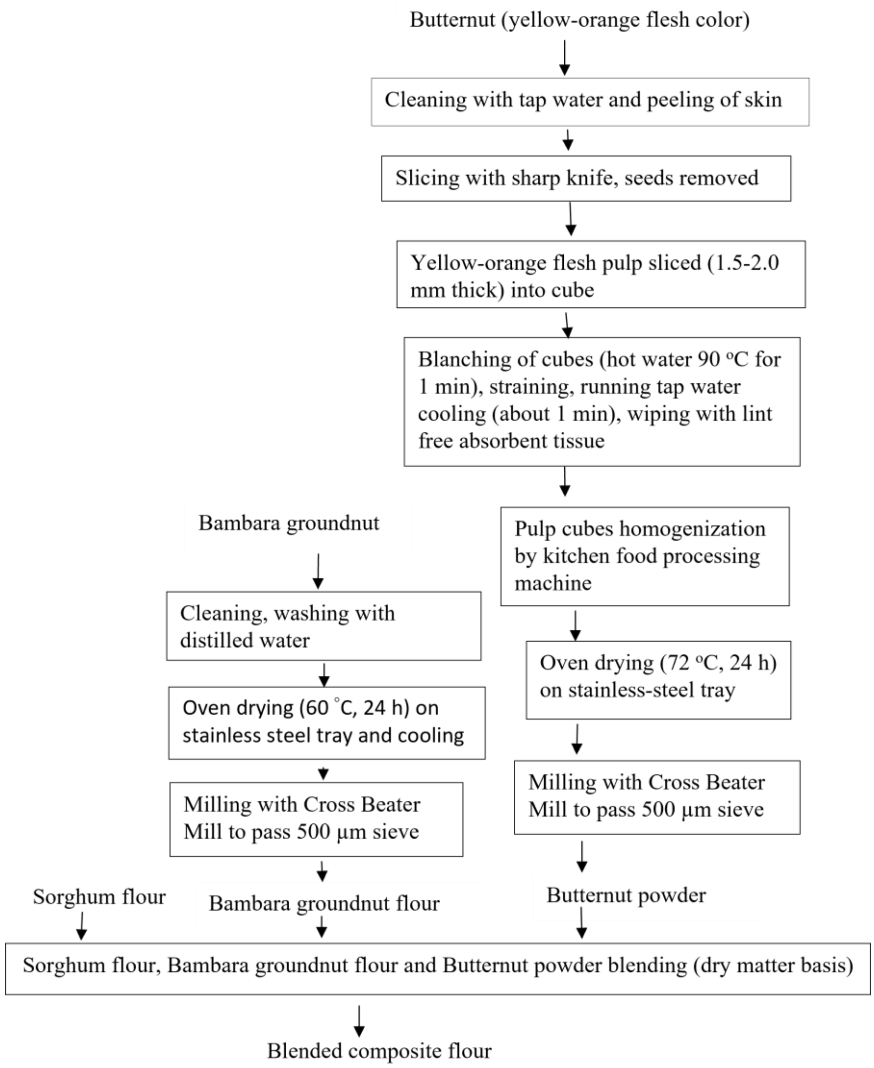


**Figure 1.** Flow diagram for production of sorghum, Bambara groundnut and butternut powder blend.

### 2.3. Experimental design

The percentages of Bambara groundnut flour, butternut powder and sorghum flour were as shown in Table 1 on a dry matter basis; 100% sorghum flour was used as the control. All of the flour samples after preparation were packed in ziplock plastic bags covered with paperboard and stored in a refrigerator (4 ℃) until used for analysis.

**Table 1.** Experimental design.

| Blends | Sorghum flour (%) | Bambara groundnut flour (%) | Butternut powder (g) |
|---|---|---|---|
| B1 | 85 | 15 | 30 |
| B2 | 75 | 25 | 30 |
| B3 | 65 | 35 | 30 |
| Control | 100 | 0 | 0 |

Note: Butternut powder was added at a constant amount (30 g/130 g × 100 = 23%).

### 2.4. Proximate composition analysis

The moisture and crude protein, fat, fiber and ash contents were determined by following AOAC method nos.: 925.10, 920.87, 920.39, 962.09 and 942.05 [24], respectively. The available carbohydrate (CHO) content was determined by difference: 100 - (% moisture + % crude protein + % crude fat + % crude fiber + % ash) [25]. The energy value (kcal/100 g) was evaluated by using the Atwater conversion factor (9 × % lipids + 4 × % proteins + 4 × % carbohydrates) [26].

### 2.5. Mineral content determination

The iron, calcium and zinc contents were determined by using atomic absorption spectrophotometry (AAS) after wet digestion of about 0.2-g samples (GBC 933 AA, GBC Scientific Equipment Pty Ltd., Australia), following AOAC method no. 968.08 [24]. The iron and zinc contents were determined via AAS by using air-acetylene as a source of energy for atomization. The absorbance for iron was measured at 248.3 nm, and the iron content was determined from the standard iron solutions calibration line (0.0 to 2.0 µg Fe/mL, $y = 14.609x + 0.0953$, $R^2 = 0.9997$) prepared from stock $FeCl_3$ in 15% HCl solution (1000 µg/mL). The absorbance for zinc was measured at 213.9 nm, and the zinc content was determined from the standard zinc solutions calibration line (0.0 to 0.8 µg Zn/mL, $y = 4.9121x + 0.0344$, $R^2 = 0.999$) prepared from the stock $ZnCl_2$ in 0.6% HCl (1000 µg/mL). For the calcium content determination, nitrous oxide-acetylene was used for atomization, and the absorbance was measured at 422.7 nm after addition of 5 mL of lanthanum solution (1%); then, the calcium content was determined from the standard calcium solutions calibration line (0.0 to 5.0 µg Ca/mL, $y = 44.847x + 0.3197$, $R^2 = 0.9953$) prepared from stock $CaCl_2$ in 6.5% HCl (1000 µg/mL).

Phosphorus content was determined after the wet digestion of an approximately 5-mg sample by measuring phosphomolybdate blue-color absorbance at 822 nm [27] from the standard phosphorus solutions calibration line (0.0 to 3.6 µg P/mL, $y = 0.0442x + 0.006$, $R^2 = 0.9776$) prepared from $K_2HPO_4$.

$$\text{Mineral element (mg/100 g)} = [(\mu g/mL \times df] / [(\text{sample mass, db}) \times 10].$$

Note: df = dilution factor (100 mL for Fe, Zn and Ca, and 20 mL for phosphorus), db = dry matter basis and 10 = conversion of 1000 g (ppm) to 100 g.

*2.6. Beta-carotene content determination*

The beta-carotene content was determined by using high pressure liquid chromatography (HPLC) (Waters Corporation, Milford, MA, USA) after extraction (2.5 g with acetone), injecting the filtrate (20 µL) into reversed-phase HPLC column (ProntoSIL Eurobond C18, 125 × 4.00 mm, 5 µm) using the mobile phase (70% acetonitrile, 20% dichloromethane and 10% methanol, flow rate: 2 mL/min) and UV-Vis detector set at 452 nm [28]. The standard beta-carotene retention time was observed from 2.70 to 2.93 min, and the sample peak retention time that matched the standard beta-carotene retention time was used. Thereafter, the beta-carotene content (mg/100 g) was determined from the calibration line (y = 50048x + 196.7, $R^2$ = 0.998) of beta-carotene standard solutions (0.0 to 5.0 µg/mL) plotted against the peak area.

$$\text{Beta-carotene (mg/100 g)} = [(\mu g/mL) \times df] / [(\text{sample mass, db}) \times 10].$$

Note: df = dilution factor (25 mL), db = dry matter basis.

*2.7. Folic acid content determination*

The folic acid content was determined by using an approximately 5-g sample after the pancreatin enzyme deconjugation treatment-assisted extraction and binding of folic acid to column that contain a gel suspension with an immunoaffinity for folic acid (P81), release of binding by solvent elution and analysis of released folic acid via HPLC [29]. The folic acid was eluted using 30% acetonitrile containing 0.2% trifluoracetic acid from the column (P81); the extract (50 µL) was injected into HPLC column (Waters Corporation, Milford, MA, USA, column: ProntoSIL Eurobond C18, 125 × 4.00 mm, 5 µm) and eluted with the mobile phase of 80% trifluoracetic acid (0.1% in water) under the following conditions: 20% acetonitrile at a flow rate of 2 mL/min; the folic acid was detected by using a UV-Vis detector set at 280 nm. The retention time for the standard folic acid solutions were observed at 4.490 to 4.543 min; the sample peak retention time that matched the standard folic acid retention time was used. The folic acid content (µg/100 g) was determined from the calibration line (y = 83551x − 375.53, $R^2$ = 0.999) of folic acid standard solutions (0.0 to 5.00 µg/mL) plotted against the peak area.

$$\text{Folic acid (µg/100 g)} = [(\mu g/mL) \times df \times 1000] / [(\text{sample mass, db}) \times 10].$$

Note: df = dilution factor (2 mL), 1000 = conversion of mg to µg, db = dry matter basis and 10 = conversion of µg/kg to µg/100 g.

*2.8. Statistical analysis*

The data generated were analyzed by performing descriptive statistics and a one-way analysis of variance using SPSS® Statistics version 25 (USA) [30], and the results are expressed as mean ± standard deviation. The mean differences were separated by using Duncan's multiple range test, and a significant difference was considered at $p < 0.05$.

## 3. Results and discussion

### *3.1. Proximate composition*

Sorghum flour improvement through food-to-food fortification has the potential to improve the nutritional quality of refined sorghum flour. The proximate composition and energy contents of composite flours from the blends of sorghum, BG flour and butternut powder are given in Table 2. The moisture contents of the flour samples ranged from 6.62% to 7.89%, and as the BG flour increased to 35%, a significant difference was observed in the moisture content ($p < 0.05$). The lowest moisture content was recorded in the flour mix where BG was 35%. High moisture content was found in the 100% sorghum flour control because of the water binding of starches that is higher in the sorghum flour [3] than BG flour [12]. The moisture contents of the flour blends were found to be below 12.0%, which is recommended for the long storage of flour, and with appropriate packaging, this can favor long shelf-life storage.

**Table 2.** Proximate composition and energy contents (dry matter basis) of sorghum (S), Bambara groundnut (BG) and butternut (BU) blends and the control sorghum flour (C).

| Blends | Moisture (%) | Protein (%) | Fat (%) | Crude fiber (%) | Ash (%) | CHO (%) | Energy (kcal/100 g) |
|---|---|---|---|---|---|---|---|
| B1 | 7.30 ± 0.10$^{b}$ | 10.34 ± 0.68$^{bc}$ | 2.59±0.03$^{c}$ | 2.09 ± 0.18$^{b}$ | 1.77 ± 0.01$^{c}$ | 75.91 ± 0.74$^{b}$ | 368.29 ± 1.07$^{c}$ |
| B2 | 7.20 ± 0.06$^{b}$ | 11.28 ± 1.20$^{b}$ | 3.09±0.13$^{b}$ | 2.11 ± 0.05$^{b}$ | 1.96 ± 0.04$^{b}$ | 74.36 ± 1.13$^{b}$ | 370.37 ± 0.31$^{ab}$ |
| B3 | 6.62 ± 0.05$^{c}$ | 14.19 ± 1.36$^{a}$ | 3.38±0.13$^{a}$ | 3.04 ± 0.28$^{a}$ | 2.19 ± 0.05$^{a}$ | 70.59 ± 1.76$^{c}$ | 369.53 ± 0.99$^{bc}$ |
| C | 7.89 ± 0.10$^{a}$ | 8.62 ± 1.16b$^{c}$ | 2.36±0.01$^{d}$ | 1.37 ± 0.28$^{c}$ | 0.87 ± 0.02$^{d}$ | 78.89 ± 1.24$^{a}$ | 371.28 ± 0.69$^{a}$ |
| Range | 6.62–7.89 | 8.62–14.19 | 2.36–3.38 | 1.37–3.04 | 0.87–2.19 | 70.59–78.89 | 368.29–371.28 |

Note: B1 = 85% S : 15% BG, B2 = 75% S : 25% BG, B3 = 65% S : 35% BG, C = 100% sorghum flour control and BU = 30 g (23%). Means in the same column followed by different superscript alphabet letters are significantly different at $p < 0.05$.

The lowest protein content (8.62%) was recorded in the control refined sorghum flour, and the highest (14.19%) was recorded in the 35% BG blended flour (Table 3). The protein contents in the blended flour significantly increased ($p < 0.05$) with BG flour increase because BG is high in protein content (17.1 to 22.9%) [9] as compared to sorghum grain flour (8.4 to 12.0%) [3]. With 35% BG blending, an increase of 37% from the control sample was observed. This shows that there is potential to improve the protein content of sorghum flour for porridge-making by adding BG flour to combat protein malnutrition, which is particularly prevalent in Sub-Saharan African children of six months to five years [31,32]. Use of BG flour can complement the limited essential amino acids like lysine, tryptophan, threonine and isoleucine [9,15], which are known to be deficient in sorghum grain [3]. In another study, an increase in the protein contents in kisra bread by fortification with BG [20] was reported. Additionally, cowpea flour fortification in sorghum flours was reported to increase the protein contents in the porridge [18].

The crude fat content in the blended flour samples ranged from 2.59 to 3.38%; it significantly increased ($p < 0.05$) as compared to that of the control sorghum flour (2.36 %) because the fat content in the BG is known to be high (6.5 to 8.5%) [9] (Table 3). The fat content of the blended flour samples was generally low, and such product may contribute to the prevention of obesity, diabetes mellitus type II and various cardiovascular disease development in humans [33].

The crude fiber content in the blended flours ranged from 2.09 to 3.04%, and with 35% BG flour blending, a significant ($p < 0.05$) increase by 45% from the control was observed (Table 3). This is because the fiber content (3.4 to 3.7%) [13] in the BG flour is high as compared to that of sorghum flour; this is because, during sorghum grain milling, the bran (pericarp, aleurone layer and germ) is removed, which is where high fiber and fat contents are concentrated. The fiber content in the blended flour samples is less than 5%, and the porridge processed from such flour can meet the Codex Alimentarius Commission [34] recommendation of less than 5% fiber in the complementary weaning foods.

**Table 3.** Mineral nutrients (Fe, Zn, Ca and P), beta-carotene and folic acid contents (dry matter basis) of sorghum (S), Bambara groundnut (BG) and butternut (BU) blends and control sorghum flour (C).

| Blends | Fe (mg/100g) | Zn (mg/100g) | Ca (mg/100g) | P (mg/100g) | Beta-carotene (mg/100 g) | Folic acid (μg/100 g) |
|---|---|---|---|---|---|---|
| B1 | $4.69 \pm 0.33^{a}$ | $2.98 \pm 0.09^{b}$ | $92.0 \pm 3.9^{c}$ | $371.07 \pm 25.87^{c}$ | $0.59 \pm 0.01^{b}$ | $0.75 \pm 0.03^{c}$ |
| B2 | $4.79 \pm 0.34^{a}$ | $3.26 \pm 0.35^{ab}$ | $100.0 \pm 0.0^{b}$ | $461.53 \pm 14.92^{b}$ | $0.59 \pm 0.02^{b}$ | $1.38 \pm 0.06^{a}$ |
| B3 | $5.08 \pm 0.16^{a}$ | $3.74 \pm 0.21^{a}$ | $106.7 \pm 5.8^{a}$ | $594.53 \pm 30.99^{a}$ | $0.63 \pm 0.03^{a}$ | $1.42 \pm 0.07^{a}$ |
| C | $3.43 \pm 0.10^{b}$ | $2.96 \pm 0.42^{b}$ | $80.0 \pm 0.0^{d}$ | $150.63 \pm 24.71^{d}$ | ND (<0.01)$^{c}$ | $0.92 \pm 0.02^{b}$ |
| Range | 3.43–5.08 | 2.96–3.74 | 80.0–106.7 | 150.63–594.53 | <0.01–0.63 | 0.75–1.42 |

Note: B1 = 85% S : 15% BG, B2 = 75% S : 25% BG, B3 = 65% S: 35% BG, C = 100% sorghum flour control; BU = 30 g (23%), Fe = iron, Zn = zinc, Ca = calcium, P = phosphorus and ND = not detected. Means in the same column followed by different superscript alphabet letters are significantly different at $p < 0.05$.

The ash content in the blended flour ranged from 1.77 to 2.19%, and it significantly ($p < 0.05$) increased with the increase in BG flour. BG flour is known to bear a higher ash content (3.6–3.84%) [13] than refined sorghum flour (0.87%) (Table 3). The lowest ash content was recorded in the control because of bran removal during sorghum grain milling. The ash content in the blended flour is in part contributed by the dried butternut powder [17].

The utilizable carbohydrate content in the blended flour (B1 = 75.91% to B3= 70.59%) decreased as the BG increased because BG is low in its carbohydrate content (57.9 to 61.7%) [13] as compared to sorghum grain flour (76.64%) [3]. A flour product with decreased utilizable carbohydrates and increased fiber contents is beneficial because a low glycemic diet is known to suppress diabetes mellitus type II disease. In this respect, BG was reported to suppress hyperglycemia and hyperlipidemia [35], which are risk factors for the development of obesity, diabetes and cardiovascular diseases [36]. Indeed, because of high soluble and insoluble dietary fibers and the associated phenolic compounds in the Bambara groundnut, its use in functional foods and as nutraceuticals are purported to suppress diabetes mellitus type II, cardiovascular diseases, and cancers [37].

The energy content in the blended flours ranged from 368.29 to 370.37 kcal/100 g (Table 3), and for the sorghum flour control (371.28 kcal/100 g), it was significantly varied from the lowest (15%)

and the highest BG (35%) blended flours. This is because the high utilizable carbohydrate content in the refined sorghum flour is reduced at the highest-level BG blending, leading to decreased energy contents. Consumption of 100 g of the blended flour in which BG is blended at 25% would contribute 17.6% of the 2100 kcal per day required for a healthy adult individual.

### *3.2. Mineral nutrient (Fe, Zn, Ca and P) content*

The iron content among the blended flours ranged from 4.69 to 5.08 mg/100 g with no significant difference ($p > 0.05$) (Table 3). But a significantly low iron content (3.43 mg/ 100 g) ($p < 0.05$) was recorded in the sorghum flour control. Blending with BG at 35% showed an increase of 48.1% iron content from the control sample; this is because iron content was found to be high in BG (5.45 mg/100 g by Abdualrahman et al. [20] and 8.8 mg/100 g by Semba et al. [38]. Iron is vital in human nutrition for the various redox reactions taking place for cellular metabolic functions; particularly, it is a component of hemoglobin and myoglobin and a co-factor for more than 200 enzymatic systems [39]. The recommended dietary allowance (RDA) of iron for a child of 1–3 years is 7 mg/day [39]. The iron bioavailability from plant-based diets can range from 5 to 10% [40], and assuming 10% bioavailability, the consumption of 100 g at 35% BG blending can supply 7.3% ($5.08 \times 10/100 = 0.508$; then, $0.508/7.0 \times 100 = 7.3\%$) of iron for a child aged 1–3 years old. This shows that, even though there is some improvement in the blended sorghum meal in terms of iron supply, other diet sources rich in iron are required for adequate iron intake along with this porridge.

Blending at 35% BG flour showed a significant increase ($p < 0.05$) in the zinc content (3.74 mg/100 g) as compared to 15% BG flour blending (2.98 mg/100 g) and the sorghum flour control (2.96 mg/100g) because the zinc content in BG is reported to be high (2.14 to 19.73 mg/100 g) [12] (Table 3). With the 35% BG flour blending, the zinc content was increased by 26.4% from the sorghum flour control zinc content. Zinc is vital in human nutrition, as it is distributed throughout all human tissues and body fluids and is indicated to be a co-factor for more than 1000 enzymatic reactions in the cellular functions, among others, that are required for normal growth and development, the regulation of gene expression and proper immune functions [41]. The RDA of zinc for a child of 1–3 years is 3.0 mg/day [39]. Considering a moderate zinc bioavailability diet (30% bioavailability that takes phytate zinc binding into account) [40], the consumption of 100 g of 35% BG blended flour porridge would supply 37.4% of the zinc ($3.74 \times 30/100 = 1.12$ mg; then, $1.12/3 \times 100 = 37.4\%$) for the daily requirement.

The calcium content for the blended samples ranged from 92.0 to 106.7 mg/100 g, and significant differences ($p < 0.05$) were observed among the BG flour blending levels (Table 3). With 35% BG flour blending, the calcium content increased by 133% from the sorghum flour control. The calcium content in the BG flour was reported as variable (0.39 to 76.01 mg/100 g) and noted as low as compared to mung beans (36.65 to 115.00 mg/100 g) and chickpeas (81.70 to 222.65 mg/100 g) [12]. For the BG grown in the Southern Africa region, a range of 37.0 to 68.0 mg/100 g calcium content was reported [42]. The calcium content of the blended flours is in part contributed from the butternut in the blend (23%), since butternut has been reported to be high in calcium content (152.34 to 244.18 mg/100 g) [43]. Calcium is the most abundant element in the human body among all others at large; it is used for bone and teeth formation and their health maintenance, muscle contractions, enzyme activation, blood clotting and neural transmissions [39]. Calcium deficiency leads to bone weakness (osteopenia) and fractures (osteoporosis); its excessive intake leads to hypercalcemia (metabolic alkalosis and loss of kidney function) and kidney stone formation [39]. The recommended adequate intake of calcium for a child of 1–3 years is 500 mg/day [39], and considering 70% calcium bioavailability [40], the consumption of 100 g of this 35% BG and 23% butternut flour blended flour can contribute 14.9% (106.67 × 70/100 = 74.7; then, 74.7/500 × 100 = 14.9%) of calcium for the daily intake.

The phosphorus content in the blended flours ranged from 371 to 594 mg/100 g. Significant variations among blending levels and difference from the control (150 mg/100 g) ($p < 0.05$) was observed because BG is reported to be high in phosphorus content (173.97 to 563.00 mg/100 g) [12] as compared to sorghum flour (278 mg/100 g) [3]. Phosphorus is widely distributed in foods. Use of phosphorus-containing additives are problematic and implicated in chronic kidney disease, hypertension, cardiovascular diseases, bone mineral soft-tissue calcification, premature aging, dementia and cancers [44]. Phosphorus is an abundant mineral in the human body at large, next to calcium, and it is found in the bones (85%) and teeth, structural components of cell membranes, parts of nucleic acids (DNA and RNA), cell energy production and energy storage (ATP) and the maintenance of buffer pH systems in the bodily fluids [39,44]. The RDA of phosphorus for a child of 1–3 years is 460 mg/day, and for 9–18 years, it is 1250 mg/100g [39]. Consumption of 100 g of 35% BG blended flour can contribute to daily phosphorus intakes of 129% and 47.5%, respectively, for children aged 1–3 and 9–18 years old. This shows that the blended flours can contribute significantly to phosphorus intake for adequate human nutrition, and that they may not pose the adverse health effects of high phosphorus intake, particularly for the population aged greater than three years, since the requirements are in the range 500 to 1250 mg/100 g per day [39].

### *3.3. Beta-carotene content*

The beta-carotene content in the blended flour samples were ranged from 0.59 to 0.63 mg/100 g (Table 3). Among the blended flours, a significant increase ($p < 0.05$) was observed for the highest BG blending, whereas, in the sorghum flour control, it was not detected (< 0.01 mg/100 g). This shows the major beta-carotene contribution to the blended flours is from the butternut flour added at 23%, in which beta-carotene content was reported as high (2.1 to 6.4 mg/100 g) [45]. Among the pro-vitamin A carotenoids, beta-carotene is known to impart high vitamin A activities (12 µg beta-carotene = 1 µg retinol activity equivalent (RAE)). Adequate intake of beta-carotene and other carotenoids are vital for normal eye vision, immune functions to fight infectious diseases and gene regulation, and as an antioxidant in the protection of cell components (DNA, RNA, proteins, and lipids) from oxidative stress damage [46]. The RDA of vitamin A for children to adults 1–3, 4–8 and 9 to > 70 years old are 0.3, 0.4 and 0.6–0.9 RAE (mg/day), respectively [39]. This shows that, for a child aged 1–3 years old, consumption of 100 g of the flour with 35% BG and 23% butternut flour blending can contribute about 17.5% of the RAE intake (0.63 mg/100 g beta-carotene = 0.0525 mg RAE, 0.0525/0.3 × 100 = 17.5%). Hence, food products produced from this flour will be beneficial in supplying pro-vitamin A carotenoids. Other carotenoids not measured in this work found in the butternut will also contribute toward RAE, and, in this respect, the contribution to RAE would be expected to be higher than this one.

### *3.4. Folic acid content*

The folic acid content in the blended flour samples ranged from 0.75 to 1.42 µg/100 g, while, for the sorghum flour control, it was 0.92 µg/100 g (Table 3). With 35% BG flour blending, a significant increase ($p < 0.05$) in the folic acid content by 154% from the refined sorghum flour control was observed. The folic acid content found in the blended samples was low as compared to the RDA (150 to 400 µg/day) for various age groups (one to >70 years), and for pregnant women (600 µg/day) [39]. Folates are a co-enzyme involved in one-carbon unit (methyl and formyl groups) transfer in cell metabolism, and they are essential, among others, in DNA and RNA synthesis protection from megaloblastic anemia, and neural tube defects (defects in the brain, spine or spinal cord) of the developing fetus [39]. In this work, only folic acid content (1 µg dietary folate equivalents = 0.6 µg folic acid) was determined. Other folacin complex compounds were not evaluated, and the potential for the blended flour to contribute to dietary folates could be higher than what was found in this result; this is because BG is a pulse grain with a reportedly high total folate content (210 µg/100 g) [38]; folate is also reportedly high in sorghum grain (25 µg/100 g) [3].

## 4. Conclusions

In this study, the blending of Bambara groundnut flour and dried butternut powder as a strategy of food-to-food fortification to overcome the nutrient limitations associated with the use of refined sorghum flour was evaluated. As the blending levels with BG were increased, protein, fat, fiber, ash, iron, zinc, calcium, phosphorus, beta-carotene, and folic acid contents significantly ($p < 0.05$) increased from those of the 100% refined sorghum flour control. The study showed that a product with improved levels of proteins, dietary fiber and micronutrients can be processed by blending 25% or 35% BG flour and 23% butternut powder into the refined sorghum flour. However, flour with a significant increase in the crude protein, crude fat, crude fiber, zinc, calcium, phosphorus, beta-carotene, and folic acid contents can be processed at 35 g of BG with 65 g of refined sorghum flour, to which 30 g (23% in the total mix) butternut powder is mixed. Further studies on changes in digestibility, micronutrient bioavailability and heat-sensitive nutrient loss as a result of cooking the flour into porridge are suggested.

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## Conflict of interest

Authors have no conflict of interest to declare.

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