

Optimization of Protein Content and Dietary Fibre in a Composite Flour Blend Containing Rice (*Oryza sativa*), Sorghum [*Sorghum bicolor* (L.) Moench] and Bamboo (*Yushania alpina*) Shoots

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How to cite this paper: Wanjala, W.N., Mary, O. and Symon, M. (2020) Optimization of Protein Content and Dietary Fibre in a Composite Flour Blend Containing Rice (*Oryza sativa*), Sorghum [*Sorghum bicolor* (L.) Moench] and Bamboo (*Yushania alpina*) Shoots. *Food and Nutrition Sciences*, **11**, 789-806.

<https://doi.org/10.4236/fns.2020.118056>

Received: March 18, 2020

Accepted: August 21, 2020

Published: August 24, 2020

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Abstract

Initiatives on tackling food insecurity among global emerging economies are being focused on enriching native staple foods with locally available nutritious underutilized crops. The objective of this study was to optimize protein content and dietary fibre in rice (*Oryza sativa*) flour using Sorghum (*Sorghum bicolor* L.) and Bamboo shoots (*Yushania alpina*). An extreme vertices design of mixture approach with 11 runs was employed in the study using MINITAB® software. The 11 blends from 11 generated runs and individual ingredient samples were analyzed for nutritional composition. Energy value and energy-to-protein ratio for the samples was calculated. Bamboo shoots flour (BSF) had the highest content for all proximate components except total carbohydrates on dry weight basis. Rice had the highest content of total carbohydrates at 77.71% and energy to protein ratio of 53.72 kcal/g. Sorghum had the highest mean total phenolic and condensed tannins of 45.512 (mg GAE/kg) and 2.512 (mg CE/g) while rice the least with 0.042 (mg GAE/kg) and 0.102 (mg CE/g), respectively. Fresh bamboo shoots had the highest level content of HCN of 117.81 mg/kg. Other dried ingredients had a mean HCN content of 2.313, 1.584 and 0.066 mg/kg for dried BSF, sorghum and rice respectively. Increasing the quantity of BSF and sorghum flour in the blends consequentially increased the protein content, dietary fibre and total minerals. Optimum blend was established to be 50:27:23 for rice, sorghum and BSF, respectively. This blend had 13.4% protein, 6.2% dietary fibre and 3.9% total minerals. Regression analysis showed that apart from dry matter, all other constituents were significantly predictable during optimization with $R^2 >$

0.7530. Cluster analysis showed that the nutritional components analyzed are in four main clusters. Cluster 1: Dry matter and protein digestibility, cluster 2: Carbohydrates, energy value and energy ratio, cluster 3: Protein, fibre and ash while cluster 4: Crude fat only. These findings of the optimum composite ratio and other blends could contribute in addressing the food insecurity for low income countries.

Keywords

Optimization, Protein, Dietary Fibre, Bamboo Shoots, Mixture Analysis

1. Introduction

Cereal crops such as corn, wheat, rice, barley and oat are global staple foods which play a key role as a major constituent in diets. These cereal and cereal based food products are very rich in carbohydrates and therefore, significantly contribute to human nutrition. However, cereal and cereal based food products are deficient in many vital nutrients such as proteins and minerals [1]. Some processing techniques associated with cereals involve removal of the germ and bran thus refined cereal products such as polished white rice and refined wheat flour [2]. Consumption of these refined cereal based food products has been associated with high glycaemic values, which is a predisposing factor to type 2 diabetes and obesity [3] [4]. It has also been reported that communities that depend primarily on cereal based foods, especially from emerging economies, suffer from health problems related to protein-energy malnutrition and micro-nutrient deficiencies [5] [6] [7]. Thus, there is a growing trend to enrich cereal and cereal based food products with proteins, fibre and minerals.

Current trends in development of nutrient dense cereal food products involve integrating plant based ingredients that are rich in the nutrients of interest. The philosophy of utilizing plant based ingredients is their assumed affordability and availability as a source of proteins, dietary fibre, minerals and phytochemicals as opposed to animal sources. The most commonly used plants for blending in order to improve on specific nutrients in deficit cereal based foods include soybeans, legume seeds, fruit pomace, gums and “pseudo cereals” such as sorghum, millet, buckwheat, quinoa and tef [8] [9]. Recent reports indicate that food blending innovations are utilizing underutilized food crops as fortificants in nutrient deficit cereal based foods. However, bamboo shoots which have been found to contain high levels of quality proteins, remain underutilized [10] in Kenya. The bamboo shoots are mainly consumed in Asian countries. Recent reports indicate that there is a significant growing demand for the bamboo shoots based foods around the world [11]. Reports on the influence of bamboo shoots incorporation on physical, sensory and technological properties of cereal based food products are scanty [12]. Thus, there is need to evaluate both the sensory and technological impact on incorporating bamboo shoots in cereal based foods.

Such information will be critical in the innovative development of new and improved food products that address the dynamic consumer demands.

Rice is one of the cereal food crops that have widely been used in processing of ready-to-eat snacks, breakfast cereals, porridge flour blends and noodles. This is because it possesses a characteristic blunt taste [13]. Many studies have focused on improving the nutritional value of rice based products by incorporating soya beans, sorghum, millet, edible insects, and peanut [14] [15] [16]. However, very scanty information is available on bamboo shoot being used to enrich rice flour and consequently rice based products. Studies have shown that bamboo is very rich in proteins, dietary fibres, minerals and phytochemicals [17] [18]. Therefore, it is proposed that preparing composite flour composed of rice, bamboo shoots and sorghum will improve both the nutritional value and physical properties of the respective food products prepared using the composite flour.

The purpose of this study was therefore, to develop nutrient dense composite flour through optimization process by standardizing the levels of bamboo shoot flour, sorghum flour and rice flour. An experimental mixture design approach was employed in order to optimize mixtures of flours. The effect of this optimization process on nutritional properties of the composites was analyzed. We hypothesized that use of sorghum and bamboo shoot flour will improve on intensity of nutrients of interest as well as other phytochemicals of the flour blends when rice is the base ingredient.

2. Materials and Method

2.1. Materials

Edible shoots of *Yushania alpina* (Alpine bamboo) were harvested from Mt. Elgon National Reserve, Kenya. This bamboo species was selected because it is a vast indigenous plant that grows wildly in Kenyan and the other East African highland areas. The shoots were harvested at 4 - 6 weeks after the onset of April-May rainfall. The husks were removed, the soft edible portions chopped into small pieces, and immediately dried by fire from wood [10]. Fresh bamboo shoots were cut into small pieces, placed in Ziploc bags and immediately transported to the laboratory in a cool box for cyanide analysis. Rice and red sorghum were procured from the local agricultural market. All the ingredients were milled using a hammer mill fitted with a sieve with pore size of <800 µm. Rice, sorghum and bamboo shoot flour were then stored in sealed polyethylene pouches.

2.2. Experimental Design

This study was carried out in two main stages: Pre-trial and trial. During pre-trial, ingredients to be used were first analyzed for their proximate composition. The protein, dietary fibre and ash were applied in mixture design to empirically set up, first proportional limits for each ingredient (constrain the ingredients) using Minitab® (Version 17.6.1, Minitab, USA). Secondly, the ingredient combination that lay outside the feasible region of optimization was screened

out. The trial stage used the constrained mixture design obtained from pre-trial to evaluate the effect of flour proportions (Rice, sorghum and bamboo shoot) on the properties of composite flour. Extreme vertices method of mixture design with constraints that comprised of Rice 0.5 - 0.7, Sorghum 0.1 - 0.3 and Bamboo Shoot Flour 0.0 - 0.3 on proportional basis so as to have a final mixture total of 1.0 (100%) was employed. Extreme vertices design was used because according to [19], this method is used in mixture experiments when constraints are applied on factors (ingredients). Hence, these factor constraints reduce the spatial volume of the factors and the factor level from 0 to 100% or 0.0 to 1.0 on proportional basis. This model generated 11 runs as shown in **Table 1**, where each run formed a composite. Each composite was analyzed in triplicates for proximate and protein digestibility. Lastly, the composition of the 11 composites was used to again empirically determine the optimum blend using the response optimizer. The proximate composition of the optimum blend was also analyzed.

2.3. Proximate Analysis

2.3.1. Dry Matter Content

The oven method of [20] Method 950.46 was used. About 2.0 g of samples was accurately weighed and transferred into aluminium dishes. The samples were dried in a dry air oven at 105°C for 24 hours and cooled in a desiccator before weighing.

2.3.2. Crude Protein

Crude protein was determined by Kjeldahl method 978.04 of [21]. Accurately 1 g of the sample was weighed into a test tube and digested with concentrated H₂SO₄

Table 1. Mixture Design extreme vertices model.

StdOrder	Optimization Order		Ingredient Ratios			Mixture Constraints		
Sample Name	Run Order	PtType	Rice	Sorghum	Bamboo	Totals	Lower	Upper
Composite 1	2	1	0.70	0.10	0.20	1.0	0.50	0.70
Composite 2	8	1	0.70	0.30	0.00		0.10	0.30
Composite 3	4	1	0.50	0.20	0.30		0.00	0.30
Composite 4	5	0	0.60	0.20	0.20			
Composite 5	6	-1	0.65	0.15	0.20			
Composite 6	7	-1	0.65	0.25	0.10			
Composite 7	9	1	0.60	0.10	0.30			
Composite 8	10	-1	0.55	0.25	0.20			
Composite 9	1	-1	0.55	0.20	0.25			
Composite 10	11	-1	0.60	0.15	0.25			
Composite 11	3	1	0.50	0.30	0.20			

Std Order = Standard order; PtType = Point type.

in presence of a catalyst until the colour turned blue. The digest was then steam-distilled using 40% NaOH to release ammonia which was trapped in a solution of boric acid. About 60 ml of the distillate was collected and titrated with 0.02 N HCl until the color changed to orange. The protein content was then calculated by multiplying the percent nitrogen content by 6.25.

$$\text{Nitrogen (\%)} = \text{N HCl} \times \frac{\text{Corrected acid volume}}{\text{Weight of sample}} \times \frac{14 \text{ g N}}{\text{Mol}} \times 100 \quad (1)$$

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25 \quad (2)$$

where: Corrected acid volume = (mL acid for sample – mL acid for blank), N HCl is Normality of HCl, 14 is the atomic weight of nitrogen and 6.25 is the conversion factor on assumption that the sample was contain 16% nitrogen.

2.3.3. *In-Vitro* Protein Digestibility (IVPD)

In-Vitro Protein Digestibility was determined using method 971.09 of [22]. Exactly 200 mg of the sample was weighed and suspended in 15 ml of 0.1 N HCl with 1.5 mg pepsin (Sigma-Aldrich, USA) in 100 ml conical flask followed by incubation at 37°C for 3 hours. The mixture was then neutralized with sodium hydroxide and treated with 4 mg pancreatin in 0.2 M phosphate buffer containing sodium azide and incubated at 37°C for 24 hrs. Trichloroacetic acid was then added to stop the reactions followed by centrifugation at 5000 g for 5 minutes. Five millilitres of aliquots were then obtained and analyzed for nitrogen content.

$$\text{IVPD (\%)} = \frac{\text{Crude protein in supernatant}}{\text{Crude protein in sample}} \times 100 \quad (3)$$

2.3.4. Crude Fat

Crude fat was determined by the Soxhlet method 934.01 of [20]. Approximately 5 g ground sample of known dry matter content was weighed accurately into an extraction thimble and covered with cotton wool. The thimble was placed into the soxhlet extractor and the fat extracted into a tared flask for 8 h using petroleum ether. The solvent was then evaporated in a rotary evaporator and the residue dried in an air oven at 105°C for 1 h before weighing.

$$\text{Crude fat (\%)} = \frac{\text{Weight dried flask with sample} - \text{weight of empty flask}}{\text{Weight of the dry sample}} \times 100 \quad (4)$$

2.3.5. Ash Content

Ash content was determined using [20] method 920.39 where 5.0 g of sample was accurately weighed and placed into crucibles. The samples were ashed in a muffle furnace at 550°C for 6 hours. The ashed samples were cooled in a desiccator to room temperature and weighed.

2.3.6. Crude Fibre

Crude fibre was determined according to [20] method 984.04. Approximately 2 g ground sample of known dry matter content was accurately weighed into a graduated 600 mL beaker. About 100 mL boiling distilled water and 2.04 M sul-

phuric acid solution added. The volume of the mixture was made up to 200 mL with boiling distilled water and maintained at this volume whilst boiling for 30 minutes on a hot plate. The mixture was then filtered using a funnel lightly packed with glass wool. The residue was washed three times with boiling distilled water. The residue and the glass wool was then transferred quantitatively back to the beaker and about 100 mL of boiling distilled water and 25 mL of 1.78 M potassium hydroxide solution added. The volume was made up to 200 mL with boiling distilled water and this volume maintained whilst boiling on a hot plate for 30 minutes. The mixture was filtered again using glass wool and was washed three times with boiling distilled water. The residue was further washed three times with small amounts of ethanol. The residue and glass wool was transferred quantitatively to a porcelain dish and dried in an air oven at 105°C for 2 hours. The sample was cooled and weighed in the porcelain dish before igniting at 600°C in a muffle furnace for 2 hours. The sample was cooled in the dish and weighed. The crude fibre content was calculated and expressed as a percentage of the sample on dry weight basis, dwb.

2.3.7. Total Carbohydrates

Total carbohydrate content was obtained by the difference between 100% and the percent sum of the values for moisture content, fat, protein, crude fibre and ash.

2.4. Total Phenolic

Total phenolic content was determined according to the method described by [23]. Exactly 400 mg of the sample was extracted with 20 ml of acidified methanol for 1 hour at room temperature. Samples were then centrifuged (Eppendorf, Hamburg, Germany) for 10 minutes at 1200 g and three replicate of the supernatants were obtained. The sample extracts (0.5 mL) were then mixed with 2.5 mL of Folin-Ciocalteu phenol reagent in a 50 mL volumetric flask and 7.5 mL of 20% (w/v). Sodium Carbonate was then added within 8 minutes after addition of the Folin-Ciocalteu reagent. The standard curve was produced using Gallic acid as standard and the total phenol content were expressed in mg of Gallic acid equivalents (GAE/kg). The contents were then mixed and the flask made up to volume with distilled water, stoppered and thoroughly mixed. Sample blanks were also carried out. The flasks were left to stand at room temperature for 2 hours after which absorbance at 760 nm was determined using UV-VIS Spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan).

2.5. Condensed Tannins

The condensed tannin content of the samples was determined by use of the Vanillin-HCl method [24]. Exactly 400 mg of the sample was extracted with 20 ml of 100% methanol for 1 hour at room temperature. Samples were then centrifuged for 10 minutes at 1200 g using a centrifuge (Eppendorf, Hamburg, Germany) set at 25°C ± 0.5°C, to obtain three supernatants. The extracts and the

vanillin reagent were maintained at $30^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ in a water bath before being mixed with the reactants. one millilitre of the sample extracts were then mixed with 5 mL of vanillin reagent in test tubes and then maintained at $30^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ in the water bath for 20 minutes. Sample blanks were also set up in which the Vanillin reagent was replaced by 4% HCl in methanol. Absorbance at 500 nm was measured using UV-VIS Spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan) with Catechin as a standard.

2.6. Hydrogen Cyanide

Hydrogen cyanide (HCN) was determined using the picrate acid paper method described by [25]. Exactly 25 mg of a sorghum, rice and freshly cut bamboo slices and flour samples were put into 0.5 ml phosphate buffer (pH 6.0) inside a small vial with a cap. A stripe of picrate paper was inserted and the vial tightly closed and incubated at room temperature for 24 hours. Liberated HCN was trapped by the picrate paper. At the end of the incubation time, the paper was removed and soaked in 10 ml of water and agitated for cyanide to dissolve in the water. Absorbance was measured at 510 nm using UV-VIS Spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan) against a blank (picrate paper incubated without sample). Total cyanide was obtained by multiplying the absorbance value by 396 which is a gradient factor observed in the normal picrate method [26].

2.7. Energy Value and Energy-to-Protein Ratio

The energy value/content of the samples was determined by multiplying the values obtained for crude protein, total carbohydrates, crude fat and dietary fibre by 4.00, 4.00, 9.00 and 2 Kcal/g respectively, and adding up the results [27]. Energy-to-protein was obtained by dividing energy value of the sample by its crude protein content.

2.8. Data Analysis

A statistical package, MINITAB, was used to generate experimental points (runs), randomize the runs, perform analysis of variance, fit the second order polynomial models and create graphical representations. Desirability index was used as a measure of assessing on how well the model obtained explains and predicts the variability observed on dependable variables. Post-hoc analysis of the experimental means employed use of Scheffe test to determine if the effect of factors was significant at $P < 0.05$. Pearson's correlation K-means cluster analysis for the dependent variables was carried out to classify them into groups of similarities.

3. Results

3.1. Nutritional Composition of the Ingredients

Nutritional parameters determined for the rice, sorghum and bamboo shoots are

shown in **Table 2**. Bamboo shoots flour had significantly higher content for all components except total carbohydrates than rice and sorghum. Rice had significantly higher content of total carbohydrates at 77.71% than that of sorghum at 65.76% and bamboo shoots at 19.27%. Rice had significantly higher energy to protein ratio of 53.72 kcal/g protein compared to bamboo shoots and sorghum. Bamboo shoots had the lowest energy value of 288.92 kcal/100 g.

3.2. Optimized Composite

Proportions of ingredients required to make a flour blend with optimum protein, dietary fibre and total minerals are shown in **Figure 1**. Blending rice, sorghum and bamboo shoots at a ratio of 50:27:23 respectively give 13.39% protein, 6.16% fibre and 3.98% total mineral contents with a composite desirability of 0.993.

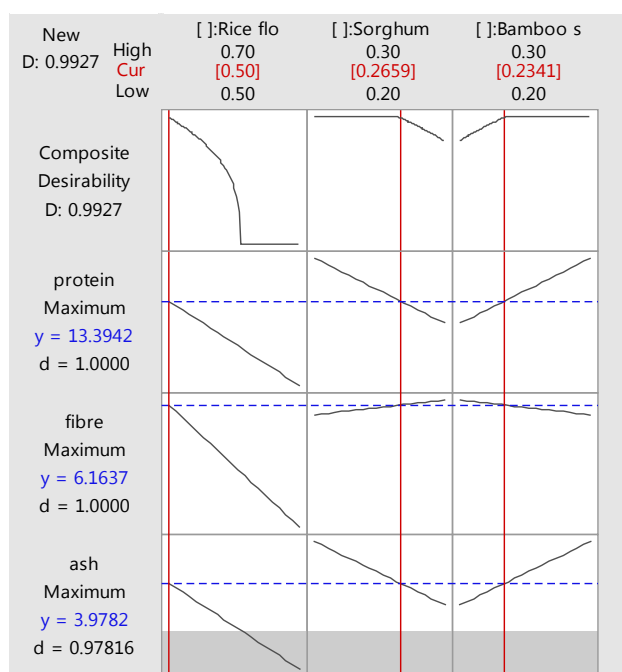


Figure 1. Optimization plots indicating best possible blend proportions for rice, sorghum and bamboo shoots that give optimum protein, dietary fibre and total minerals. Key: D = Overall experimental desirability (same as coefficient of determination); d = Each component desirability; y = Quantity of response.

Table 2. Nutritional composition for rice, sorghum and bamboo shoots used in the optimization process.

Ingredient	Dry Matter (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)	Crude Fibre (%)	Total CHOs (%)	PD (%)	EV (Kcal/100 g)	ER (Kcal/g of Protein)
Bamboo	89.89 ± 0.37 ^a	27.31 ± 0.17 ^a	6.14 ± 0.40 ^a	16.27 ± 0.04 ^a	23.66 ± 0.14 ^a	19.27 ± 2.74 ^c	63.58 ± 1.06 ^b	288.92 ± 7.37 ^b	10.58 ± 0.30 ^c
Rice	86.35 ± 0.18 ^b	6.47 ± 0.32 ^c	0.85 ± 0.13 ^c	0.29 ± 0.02 ^c	1.34 ± 0.28 ^c	77.71 ± 0.59 ^a	85.61 ± 0.83 ^a	345.79 ± 0.75 ^a	53.72 ± 2.51 ^a
Sorghum	89.49 ± 0.03 ^a	9.65 ± 0.01 ^b	3.61 ± 0.03 ^b	1.82 ± 0.05 ^b	8.65 ± 0.18 ^b	65.76 ± 0.22 ^b	63.90 ± 0.61 ^b	351.43 ± 0.27 ^a	36.43 ± 0.01 ^b

CHOs = Carbohydrates; PD = Protein Digestibility; EV = Energy Value; ER = Energy to protein ratio; Means with the letter along the column are not significantly different at P < 0.05.

Graphical representation of the sweet spot which shows region of optimum blend and contribution of each ingredient to protein, fibre and total minerals in the composite blends during optimization is shown in **Figure 2**. It was found that increasing proportions of bamboo shoot in the composite blend immensely increased protein content and total minerals. Both bamboo shoots and sorghum contributed in the increase of dietary fibre.

3.3. Nutritional Composition of Individual Runs

Nutritional composition of composite blends used in the optimization process is shown in **Table 3**. Composite 2 which was 70:30 for rice to sorghum, respectively had the highest carbohydrates content of 73%, while the energy value of 346 kcal/100 g and energy to protein ratio of 52.28 kcal/g of protein were observed. Composite 3 that was 50:20:30 for rice, sorghum and bamboo shoots respectively, had the highest content of protein of 13.6%, while the fibre content of 10.94%

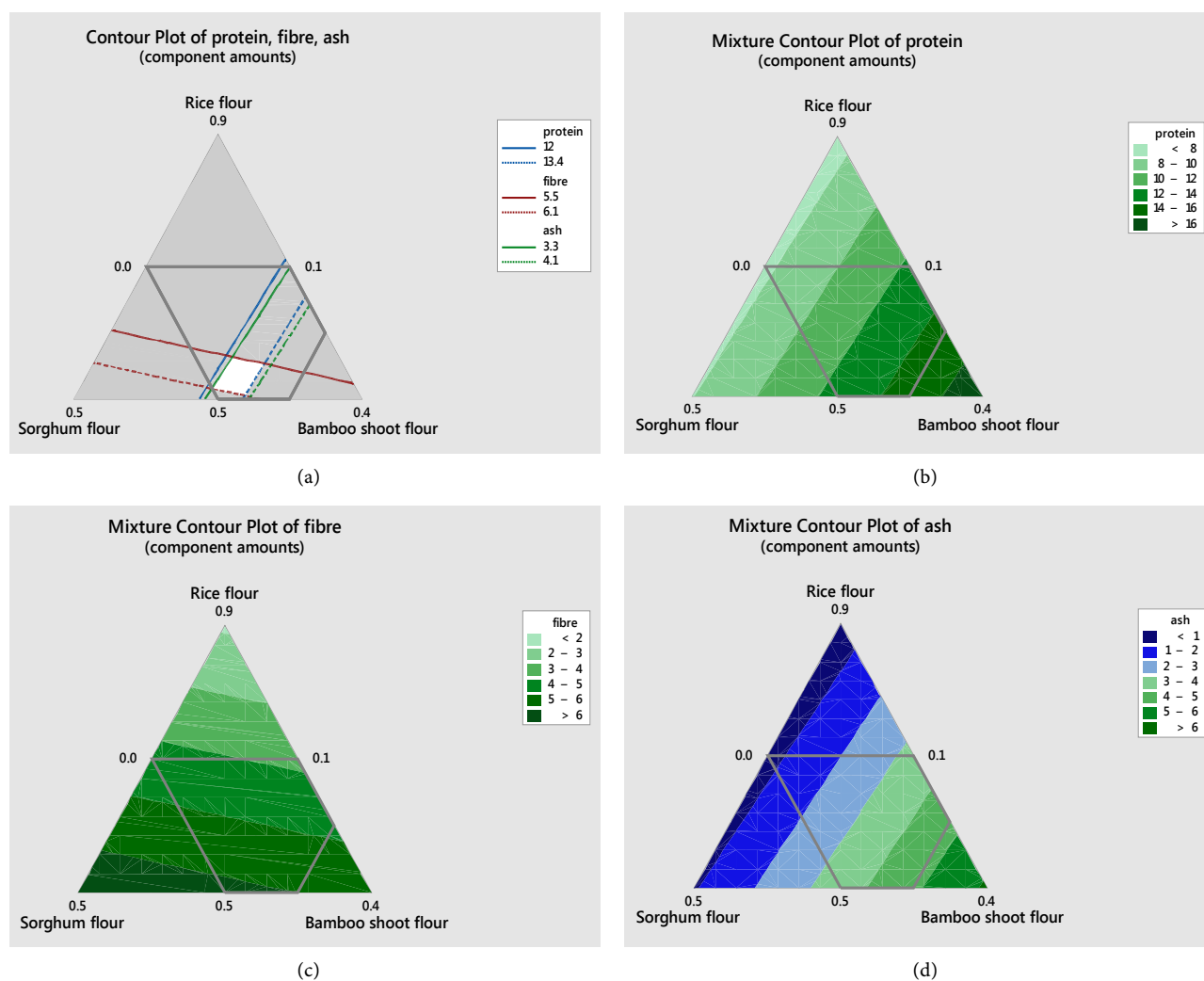


Figure 2. Overlaid contour plots of the effect of mixture components. (a) Sweet spot region indicating where optimum blend lies; (b) Contribution of each ingredient used in the blending to protein content; (c) Contribution of each ingredient used in the blending to fibre content; (d) Contribution of each ingredient used in the blending to total minerals content.

Table 3. Nutritional composition of the different blend composites used during optimization.

Sample Name	Dry Matter (%)	Ash (%)	Crude Protein (%)	Crude Fibre (%)	CHO (%)	PD (%)	Crude Fat (%)	EV (Kcal/100 g)	ER (Kcal/g of Protein)
Composite 1	89.74 ± 0.20 ^a	3.67 ± 0.06 ^c	10.70 ± 0.18 ^d	8.01 ± 0.18 ^d	65.73 ± 0.17 ^b	65.65 ± 0.19 ^a	1.63 ± 0.08 ^b	336.44 ± 0.88 ^c	31.48 ± 0.60 ^c
Composite 2	87.94 ± 0.23 ^b	0.76 ± 0.01 ^e	6.60 ± 0.20 ^f	5.66 ± 0.01 ^e	73.11 ± 0.06 ^a	60.08 ± 0.58 ^c	1.82 ± 0.01 ^a	346.49 ± 0.91 ^a	52.58 ± 1.38 ^a
Composite 3	89.16 ± 0.33 ^{ab}	5.37 ± 0.01 ^a	13.60 ± 0.08 ^a	10.94 ± 0.05 ^a	57.42 ± 0.23 ^d	61.49 ± 0.31 ^{bc}	1.84 ± 0.02 ^a	322.49 ± 1.13 ^{de}	23.71 ± 0.06 ^c
Composite 4	89.49 ± 0.07 ^a	3.79 ± 0.01 ^c	11.37 ± 0.01 ^c	8.86 ± 0.02 ^{cd}	63.76 ± 0.09 ^{bc}	64.35 ± 0.19 ^{ab}	1.70 ± 0.01 ^b	333.59 ± 0.26 ^c	29.33 ± 0.04 ^d
Composite 5	89.10 ± 0.13 ^{ab}	2.99 ± 0.37 ^c	10.21 ± 0.51 ^{de}	7.76 ± 0.25 ^d	66.45 ± 0.95 ^b	64.85 ± 0.41 ^a	1.69 ± 0.05 ^b	337.38 ± 1.70 ^c	33.22 ± 1.73 ^c
Composite 6	88.69 ± 0.08 ^{ab}	1.89 ± 0.19 ^d	8.57 ± 0.31 ^e	6.81 ± 0.23 ^{de}	69.69 ± 0.66 ^{ab}	62.41 ± 0.37 ^b	1.74 ± 0.02 ^{ab}	342.27 ± 0.81 ^{ab}	40.06 ± 1.50 ^b
Composite 7	89.36 ± 0.24 ^a	5.32 ± 0.04 ^a	13.37 ± 0.02 ^{ab}	9.15 ± 0.35 ^b	59.73 ± 0.05 ^d	62.23 ± 0.26 ^b	1.78 ± 0.11 ^a	326.76 ± 0.21 ^d	24.43 ± 0.06 ^e
Composite 8	89.70 ± 0.18 ^a	3.40 ± 0.23 ^c	10.78 ± 0.39 ^{cd}	8.20 ± 0.62 ^d	65.64 ± 1.50 ^b	60.04 ± 0.17 ^{cd}	1.69 ± 0.08 ^b	337.27 ± 2.50 ^c	31.39 ± 1.31 ^c
Composite 9	88.81 ± 0.11 ^{ab}	4.52 ± 0.03 ^{ab}	12.47 ± 0.05 ^b	9.74 ± 0.10 ^{ab}	60.94 ± 0.29 ^{cd}	61.69 ± 0.02 ^b	1.80 ± 0.02 ^a	329.31 ± 1.10 ^d	26.41 ± 0.16 ^c
Composite 10	87.24 ± 0.03 ^b	4.48 ± 0.03 ^b	10.42 ± 0.22 ^{de}	9.03 ± 0.23 ^{bc}	61.72 ± 0.02 ^c	62.01 ± 0.10 ^b	1.59 ± 0.03 ^b	320.93 ± 0.39 ^c	30.82 ± 0.60 ^{cd}
Composite 11	88.55 ± 0.20 ^{ab}	3.91 ± 0.03 ^{bc}	11.71 ± 0.41 ^{bc}	9.01 ± 0.11 ^c	61.83 ± 0.09 ^c	58.04 ± 0.29 ^d	2.08 ± 0.06 ^a	330.94 ± 0.62 ^{cd}	28.34 ± 0.93 ^{de}

CHOs = Carbohydrates; PD = Protein Digestibility; EV = Energy Value; ER = Energy to protein ratio; Means with the letter along the column are not significantly different at $p < 0.05$.

and total minerals of 5.37% were observed. This blend exhibited the lowest content of carbohydrates of 57%, while the energy value of 322.49 kcal/100 g and energy to protein ratio of 23.71 kcal/g of protein were observed.

3.4. Effect of Optimization Process on Mixture Components

Regression coefficients for the proximate composition due to contribution mixture components are shown in **Table 4**. It was observed that apart from dry matter, all other constituents are significantly predictable when making a flour blend from rice, sorghum and bamboo shoots. Total minerals content had a higher predictability with $R^2 = 0.9553$ while crude fibre had the least with $R^2 = 0.7530$.

Cluster groups of dependent variables based on Pearson's correlation coefficients is shown in **Figure 3**. It was observed from the dendrogram that the nutritional components analyzed are in four main clusters. Cluster 1 had dry matter and protein digestibility, cluster 2 had carbohydrates, energy value and energy ratio, cluster 3 had protein, fibre and ash while cluster 4 had crude fat only.

3.5. Phytochemical Content

Total phenolic and condensed tannin content for bamboo shoot, rice and sorghum are shown in **Table 5**. Sorghum had significantly higher mean total phenolic and condensed tannins of 45.512 (mg GAE/kg) and 2.512 (mg CE/g) respectively as compared to rice and bamboo. Rice had extremely low mean total phenolic and condensed tannins of 0.042 (mg GAE/kg) and 0.102 (mg CE/g), respectively.

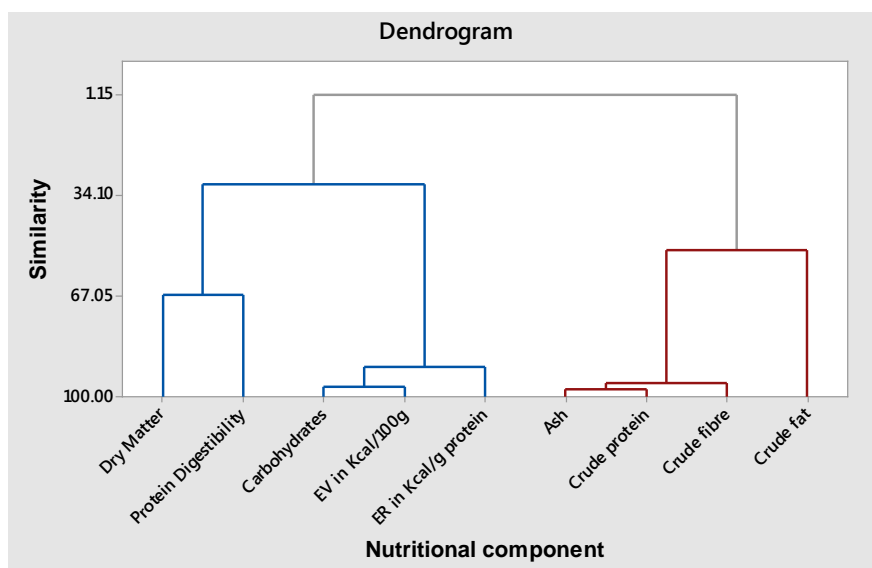


Figure 3. Cluster groups for nutritional parameters in composite blends from rice, sorghum and bamboo shoot flours based on their similarities.

Table 4. Predicted regression model equations.

Parameter	Predicted regression model***	R ²
Crude protein	$Y = 10.36X_1 + 38.33X_2 + 46.77X_3$	0.8981
Ash	$Y = 1.62X_1 + 16.01X_2 + 27.60X_3$	0.9553
Crude fibre	$Y = 12.2X_1 - 8.75X_2 + 51.10X_3 - 78.52X_1X_3 + 54.39X_2X_3$	0.9028
Carbohydrates	$Y = 67.83X_1 + 29.97X_2 - 43.53X_3$	0.9353
Crude fat	$Y = 2.31X_1 + 13.33X_2 + 4.71X_3 - 18.04X_1X_2 - 14.50X_1X_3$	0.7530

X₁, X₂ and X₃ were the mixture components; Rice, Sorghum and Bamboo shoot flours respectively; R² = Coefficient of determination; ***backward elimination regression procedure was used and non-significant terms at $p < 0.05$ were removed from the equations.

Table 5. Total phenolic content and condensed tannin of the ingredients.

Ingredient	Total phenolic content (mg GAE/kg)	Condensed tannins content (mg CE/g)
Bamboo shoot	32.792 ± 0.723^b	2.052 ± 0.027^b
Rice	0.042 ± 0.002^c	0.102 ± 0.003^c
Sorghum	45.512 ± 0.001^a	2.512 ± 0.001^a

GAE = Gallic Acid Equivalent; CE = Catechin Equivalent; Std = Standard. Means with the letter along the column are not significantly different at $P < 0.05$.

Hydrogen cyanide (HCN) content in sorghum, rice, dried bamboo shoots and fresh bamboo shoots is shown in **Figure 4**. Fresh bamboo shoots had highest level content of HCN of 117.81 mg/kg. Other dried ingredients had a mean HCN content of 2.313, 1.584 and 0.066 mg/kg for dried bamboo, sorghum and rice respectively.

Total phenolic, condensed tannin and HCN content of composite blends used in the optimization process is shown in **Table 6**. It was found out that increasing the proportion of rice in the blend significantly reduced total phenolic and HCN content. Composite 2 which was 70% rice and 30% sorghum had the lowest mean content of total phenolic and HCN at 9.308 mg GAE/kg and 0.318 mg/kg.

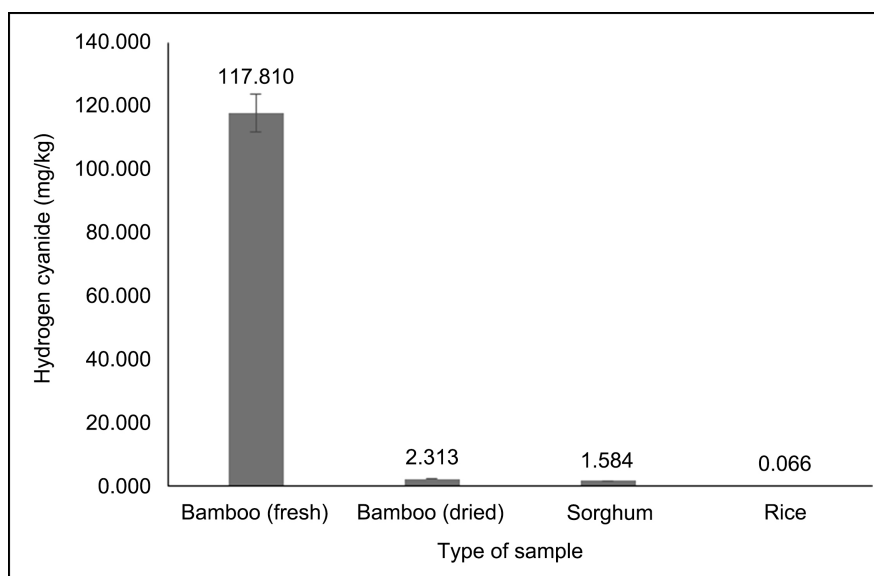


Figure 4. Hydrogen cyanide content in ingredients used in the optimization and fresh bamboo shoots.

Table 6. Total phenolic, condensed tannin and hydrogen cyanide of the different blend composites used during the optimization.

Flour blend	Total phenolic content (mg GAE/kg)	Condensed tannins content (mg CE/g)	Hydrogen cyanide (mg/kg)
Composite 1	7.779 ± 0.022 ^g	0.628 ± 0.006 ^e	0.412 ± 0.002 ^e
Composite 2	9.308 ± 0.102 ^f	0.670 ± 0.023 ^{de}	0.318 ± 0.003 ^f
Composite 3	12.380 ± 0.015 ^b	1.048 ± 0.015 ^{bc}	0.672 ± 0.001 ^a
Composite 4	10.838 ± 0.001 ^d	0.860 ± 0.042 ^c	0.528 ± 0.001 ^c
Composite 5	9.362 ± 0.031 ^f	0.768 ± 0.007 ^d	0.487 ± 0.007 ^d
Composite 6	10.352 ± 0.234 ^{de}	0.755 ± 0.001 ^d	0.425 ± 0.000 ^e
Composite 7	10.048 ± 0.001 ^e	0.813 ± 0.003 ^d	0.582 ± 0.008 ^b
Composite 8	12.333 ± 0.057 ^b	1.094 ± 0.001 ^b	0.573 ± 0.001 ^b
Composite 9	11.675 ± 0.018 ^c	1.539 ± 0.016 ^a	0.567 ± 0.002 ^b
Composite 10	10.386 ± 0.029 ^d	0.819 ± 0.010 ^{cd}	0.535 ± 0.001 ^c
Composite 11	13.942 ± 0.001 ^a	1.130 ± 0.054 ^b	0.682 ± 0.000 ^a

GAE = Gallic Acid Equivalent; CE = Catetchin Equivalent; Means with the letter along the column are not significantly different at $P < 0.05$.

4. Discussion

Rice has high levels of carbohydrates that contribute to energy values in the diets as shown in **Table 2**. However, rice is a poor source of other assessed nutrients that were of interest in this study. For example, rice has a very low content of dietary fibre, disposing it to high glycaemic indices. Considering that rice is a major staple food for many communities around the world, over reliance on it as a food predisposes the respective consumers to malnutrition. According to [28] it is estimated over 3 billion worldwide consume rice as a staple food. Studies have linked consumption of rice with both macronutrient and micronutrient deficiencies [29] [30] [31]. Therefore, the call to enrich rice based food products is vindicated. Conversely, according to [13] rice has bland taste, attractive white colour, hypoallergenicity and ease of digestion which is a desired functional property when developing a nutrient dense flour blend.

On other hand, sorghum a cereal crop is of high in most of the nutritional parameters of interest as shown in **Table 2**. Total carbohydrates in sorghum contribute to about 75% of the energy value, while protein, fat and fibre are contributing 11%, 9% and 5%, respectively. However, sorghum remains to be one of nutritious foods that is still underutilized [32] [33]. There is a growing trend among developing countries advocating for consumption of sorghum as a measure to address food security. Sorghum is a drought tolerant crop and therefore better placed in fighting food insecurity. As a result, sorghum is heavily being used to enrich other food crops in addressing protein-energy malnutrition and micronutrient deficiencies [34] [35] [36].

Unlike rice and sorghum, bamboo shoots are rich in protein, fibre and total minerals as shown in **Table 2**. Carbohydrates contribute about 27% of energy in bamboo shoots compared to protein and fibre that contribute 38% and 17%, respectively. Thus, incorporating bamboo shoots and sorghum into rice is anticipated to not only improve on nutrient density but also increase their consumption. This might explain why bamboo shoot consumption is being fronted as the next niche in tackling food insecurity due to the climate change food security challenges [37].

As shown in **Figure 1**, optimum protein, fibre and total minerals was established to be from a composite blend ratio of 50:27:23 for rice, sorghum and bamboo shoots, respectively. This optimum blend has about 106%, 350% and 1200% more protein, fibre and total minerals than rice. Graphically, optimum composite blend is indicated as a white region of **Figure 2(a)**. The region is usually referred to as sweet spot. This sweet spot is a result of considering nutrient composition of each ingredient contributing to the overall blend. As shown **Figure 2(b)**, bamboo shoot contributed more of protein than sorghum while rice contribution was almost negligible. A similar trend is observed in **Figure 2(d)** for total minerals. However, it was observed that sorghum and bamboo shoots had almost equal contribution to dietary fibre in the composite blend as shown in **Figure 2(c)**. These findings of the optimum composite ratio

could contribute in addressing the major dietary risks for low income countries. According to [38], consumption of diets low in whole grains and low in vegetables were identified as major dietary risks in low income countries. Intakes of about 25 - 29 g per day of dietary fibre could confer even greater benefit to protect against cardiovascular diseases, type 2 diabetes, and colorectal and breast cancer [39].

Besides graphical representation of how and where the optimum blend lies in the mixture design, composition of each composite shown in **Table 3** helps to understand how varying ingredients proportions affected nutrient density during optimization. Increasing the proportion of bamboo shoot had a negative implication on the energy value and energy to protein ratio of the composite blend. Regression analysis, shown in **Table 4**, indicates that the contribution of each ingredient to proximate composition of the blends was synergistic except bamboo's contribution to carbohydrates. It can be attributed to the fact that bamboo shoots are low in carbohydrates compared to rice and sorghum. Interaction between sorghum and bamboo shoots was also synergistic for crude fibre. However, interaction between rice and sorghum contributed negatively for crude fibre and crude fat in the composite blends. Similarly, the interaction between rice and sorghum contributed to crude fat. All interactions for protein, total minerals and carbohydrates were not significant at $p < 0.05$.

Cluster analysis was carried out to classify nutritional properties on the basis of similar contribution based on their degree of association during the optimization as shown in **Figure 3**. The three nutritional parameters under study: Protein, fibre and total minerals were observed to be greatly associated, in cluster 3. This implies that optimization of macronutrients to enrich rice using sorghum and bamboo shoots also greatly affect the micronutrient composition. Therefore, these three ingredients used in the optimization can be used not only to address protein-energy malnutrition but also micronutrient deficiencies. Cluster 1 featured dry matter and protein digestibility which are the only components that could be predicted during optimization as shown by regression analysis in **Table 4**. While cluster 2 had carbohydrates, energy value and energy-to-protein ratio which were the major nutritional parameters in base ingredient, rice. This means that the optimization process which was increasing nutrient intensity in composite blends affected cluster 2 by lowering their respective values. Lastly, cluster 4 had only crude fat. This is because all ingredients used are poor sources of fat in human diets.

Besides nutritional components, this study also considered the effect of optimization on some phytochemicals. Both sorghum and bamboo shoots showed to be good sources of total phenolic compounds and condensed tannins as compared to rice as shown in **Table 5**. Reducing the rice proportion resulted in increase of phenolic compounds and condensed tannins in the composite blend during optimization as shown in **Table 6**. Presence of these phenolic compounds have been known to affect the sensory (appearance, taste

and aroma) and oxidative properties of a food. Condensed tannins also called proanthocyanidins are known to bind proteins, carbohydrates and minerals thus decreasing their availability/digestibility. Phenolic compounds and tannins in foods have been associated beneficial health effect such as being anti-oxidants, cholesterol-lowering, anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardio-protective and vasodilatory properties [40] [41]. Thus, it can be inferred that sorghum and bamboo shoots also enriched rice with compounds that possess these functional properties.

Bamboo shoots are reported to contain very high levels of hydrogen cyanide [42] [43]. In the current study, fresh bamboo shoots contained a mean of 117.81 mg/kg HCN as shown in **Figure 4**. However, drying process of the shoots resulted in about 98.3% reduction to 2.313 mg/kg HCN content. Rice contained only trace amounts of HCN at 0.066 mg/kg. Similar to phenolic compounds and condensed tannins, reducing the rice proportion in the composite blends during optimization resulted in effective increase of HCN as shown in **Table 6**. It is anticipated that further processing of the flour blends will reduce the HCN to levels even further.

5. Conclusion

The findings of the current study ascertain the hypothesis that utilization of sorghum and bamboo shoot to enrich rice could be an alternative strategy to develop nutrient dense flour. Adoption of this strategy would be worth considering the different enriched flour blends, besides the optimized blend, could be used for processing in domestic and industrial applications. Although, freshly harvested bamboo shoots contained very high contents of hydrogen cyanide, the drying process over a fire place greatly reduced the contents to a safe level. This means that composite blends that contain bamboo shoot flours are safe in relation to the risk of cyanide poisoning. Further thermal processing methods that could be applied on the composite blends are expected to eliminate the cyanides. Red sorghum on other hand influenced the colour of the composites.

Acknowledgements

This study was funded through a research grant from the African Development Bank (AfDB) in collaboration with the Center of Excellence for Sustainable Agriculture and Agribusiness Management (CESAAM), Egerton University, Kenya.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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