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# In-Vitro protein digestibility, physico-chemical properties and nutritional quality of sorghum-green gram cookies supplemented with mango powder

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### ABSTRACT

Ready to Eat (RTE) sorghum cookies were prepared by incorporating green gram flour at 10%, 20%, 30%, dried mango powder at 10% and evaluated for their physico-chemical and nutritional properties. Protein, fat, fiber and ash increased with increase in green gram flour substitution as carbohydrate content decreased significantly. Significant differences ( $p \leq 0.05$ ) in protein content were seen in cookies ranging from 9.52% to 13.60%. Fiber increased significantly from 9.40% to 10.90%. In vitro protein digestibility ranged from  $67.75 \pm 0.01\%$  to  $90.05 \pm 0.10\%$ . Vitamins analysed increased with addition of green gram flour. Thiamine content ranged from  $0.22 \pm 0.02$  to  $0.61 \pm 0.02$  mg/100g, riboflavin from  $0.09 \pm 0.00$  to  $1.39 \pm 0.04$  mg/100g and ascorbic acid from  $13.87 \pm 0.79$  to  $19.31 \pm 0.94$  mg/100g. Value addition of under-utilized crops like sorghum and green grams can play a vital role in development of high nutritional quality RTE products.

**Keywords:** In-Vitro protein digestibility, physico-chemical, nutritional quality, ready-to-eat, sorghum, cookies.

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## 1.0 Introduction

Food and Nutrition Security is still a big challenge particularly to underprivileged population groups leading to the Food and Agriculture Organization estimating 800 million people are still food insecure (Baldermann et al. 2016). Preliminary assessment by the FAO has shown an additional 83 to 132 million people to the total number of undernourished people globally in 2020 especially due to the COVID-19 pandemic which has caused food supply disruptions (FAO 2020). Malnutrition is the deficiency of nutrients as a result of not ingesting the proper nutrients or consumption of nutrient-poor food with regards to the daily nutritional requirements (Baldermann et al. 2016). It is an atrocious dilemma which is affecting the masses whose diet is comprised of cereal based, starchy foods. Iron deficiency anemia, mental impairments, vitamin A deficiency, blindness, chronic diseases like cancer, cardiovascular diseases as well as diabetes are associated with malnutrition due to unbalanced diet (Baldermann et al. 2016). With diet-related non communicable diseases such as overweight and obesity being on the rise, major shifts in the production and consumption of food to improve diet quality and reduce food insecurity need to be explored further to address these other forms of malnutrition.

Cookies are ready-to-eat convenient foods categorized as snacks which appeal to a wide range of population due to their taste, longer shelf-life as compared to bread and their relatively low cost (Yusufu et al. 2016). According to various studies, snack consumption has been on the rise as a result of urbanization, cookies representing the largest category among baked food products (Onwurafor et al. 2019). Cookies being cereal-based are a good source of carbohydrate and are predominantly made from wheat. They are however lacking in other essential nutrients such as proteins and vitamins hence the many attempts to improve their nutritive value through modifying their nutritional composition.

Improving their nutritive value has led to the use of non-wheat flour or composite flour from local underutilized crops with high protein content to overcome the dependence on wheat importation especially in developing countries (Yusufu et al. 2016).

The nutritional quality of sorghum (*Sorghum bicolor* (L) Monench) and protective health effects against certain chronic diseases have been explored through food products that incorporate sorghum (Wu et al. 2018). Phenolic compounds and antioxidant activity of sorghum which are both correlated to each other offer these protective health effects. Earlier studies have shown the antioxidant activity and anticarcinogenic effects of sorghum grains which can help mitigate risks of cardiovascular diseases (De Petre et al. 2016). Green grams (*Vigna radiata*) are legumes with a high nutritional quality including being rich in proteins, vitamins (A, B1, B2, niacin) and minerals such as phosphorus, potassium, and calcium (Nanyen et al. 2016). Due to their nutritive composition, green grams can be utilized in baked products to offer healthy nutritious products. Legumes have been used in food products to improve the quality of cereal protein by supplementing with limiting amino acids in cereals (Nanyen et al. 2016).

The development of cookies from cereal-legume-fruit flour blend could provide healthy nutritious and gluten free products which in turn would provide solutions to food insecurity and malnutrition problems through diversification of under-utilized crops. The present study therefore aimed to develop gluten free cookies from sorghum and green grams supplemented with mango powder acceptable to consumers.

## 2.0 Materials and methods

### 2.1 Sample Collection

Sorghum (*Sorghum bicolor* (L) Monench), green grams (*Vigna radiata*) and ripe mangoes (*Mangifera indica* L.) were purchased from a local open-air market in Kangemi, Nairobi-Kenya. All the other ingredients (margarine,

sugar, skimmed milk powder, vanilla essence etc) used in the baking of the cookies were purchased from the local retail outlets. All materials were transported to the department of Food Science, Nutrition and Technology at the University of Nairobi, Kenya where the study was conducted.

## **2.2 Malting of Sorghum**

Sorghum (*Sorghum bicolor (L) Monenich*) was manually cleaned to remove damaged grains, husks and stones. To suppress mold growth, the grains were washed in 5% (w/v) sodium chloride solution after which they were steeped in tap water in ratio 1:2 (w/v) at room temperature ( $32 \pm 2^{\circ}\text{C}$ ) for 12 hours. After steeping, water was drained and the grains were allowed to germinate between two damp cloths at ambient temperature for 72 hours as described by Ouazib et al. (2016). After every 12-hour interval, water was sprinkled to facilitate the germination process. The germination time and the temperature chosen for this study were chosen from previous studies. At the end of the germinating period, the grains were dried in the oven at  $65^{\circ}\text{C}$  for 9 hours followed by removal of the dried sprouts on the grains manually (Siddiqua et al. 2019). The germinated sorghum grains were milled into flour using a hammer miller and passed through a 1mm sieve to obtain fine sorghum flour of uniform particle size free from clumps. The obtained flour was stored in an airtight container stored at room temperature for further analysis.

## **2.3 Fermentation of Sorghum**

Sorghum (*Sorghum bicolor (L) Monenich*) was sorted manually to remove damaged seeds and stones. The grains were then washed and dried then milled into flour using a hammer mill. The milled flour was subjected to spontaneous fermentation by microflora (lactic acid bacteria) present in the grains. Sorghum flour was fermented according to the method described by Elkhalifa et al. (2017). Flour was mixed with water (1:2 w/v) to form a slurry which was incubated at  $37^{\circ}\text{C}$  for 48hrs in sterile covered flask. After the fermentation period, excess

water was removed and the fermented slurry was oven dried at  $65^{\circ}\text{C}$  for 8hrs and then ground into flour. The fermented ground flour was passed in a 0.4mm sieve and stored in an airtight container until needed for analysis.

## **2.4 Preparation of Whole Grain Sorghum Flour**

Sorghum (*Sorghum bicolor (L) Monenich*) was sorted out to remove excess dirt and thoroughly washed as per the method reported by Adeyeye 2016. The cleaned grains were oven dried at  $65^{\circ}\text{C}$  for 8 hours then milled using a commercial hammer mill into fine flour. The dried flour was packaged in a clean airtight container for further use.

## **2.5 Preparation of Green Gram Flour**

Green grams (*Vigna radiata*) flour was produced using the method of Dabels et al. (2016). The grains were manually cleaned to remove all the dirt then washed with clean water. The clean seeds were then spread on tray and placed on a drying rack to dry for 4 hours. The dried green grams were then milled into flour using a commercial hammer miller. The flour was then passed through a 1mm sieve and packed in a clean airtight container for further use.

## **2.6 Drying of Mango (*Mangifera indica L.*) Fruits**

In preparation of the dried mango powder, the method of Sengev et al. (2015) was adopted with slight modifications. Twenty large-sized ripe mangoes bought from the market were sorted out, washed, peeled and the mesocarp was sliced manually to a thickness of 2.0mm. The thin slices were spread on a tray lined with aluminium foil and oven dried at  $60^{\circ}\text{C}$  for 24 hours. The dried slices were then ground into powder using a grinder and sieved through a 0.5mm sieve to obtain the dried mango powder that was stored in an airtight container stored at room temperature.

## **2.7 Experimental Design and Sample Formulation**

The experiment consisted of a  $3 \times 4$  factorial design with three levels of sorghum flour (whole

grain, malted, fermented) and four levels of green gram flour and dried mango powder substitution (100:0:0, 80:10:10, 70:20:10, 60:30:10) for each of the treatments. The ratio of the flour blends was labelled CLF (Cereal: Legume: Fruit) to represent the sorghum: green gram: mango powder ratio in the formulations.

Sample CLF1 with 100% whole grain sorghum flour served as the control sample. Formulation (**Table 2.2**) was done based on the recommended minimum levels of protein, fiber, fat by FAO/WHO which were achieved using NutriSurvey Linear Programming Package 2004 version.

**Table 2.1: Formulation of Ingredients Used in the Sorghum-Green Gram Cookies**

Ingredients	Amount (grams)
Flour *	200
Sugar	50
Margarine	50
Salt	0.35
1 Whole Egg	30
Skimmed Milk Powder	8
Baking Powder	1.25
Vanilla Essence	1.25
*Sorghum flour, green gram flour and dried mango powder	

**Table 2.2: Ratio of the Flour Blends**

Flour Blends	Whole-Grain Sorghum Flour (%)	Malted Sorghum Flour (%)	Fermented Sorghum Flour (%)	Green Gram Flour (%)	Dried Mango Powder (%)
CLF 1	100	0	0	0	0
CLF 2	80	0	0	10	10
CLF 3	70	0	0	20	10
CLF 4	60	0	0	30	10
CLF 5	0	100	0	0	0
CLF 6	0	80	0	10	10
CLF 7	0	70	0	20	10
CLF 8	0	60	0	30	10
CLF 9	0	0	100	0	0
CLF 10	0	0	80	10	10
CLF 11	0	0	70	20	10
CLF 12	0	0	60	30	10

**Key: CLF = Cereal: Legume: Fruit Blend**

### 2.8 Formulation of the RTE Cookies

The ingredients used in the sorghum-green gram cookie preparation included the different ratios of the sorghum flour (whole grain, malted and fermented), green gram flour and mango fruit powder. The cookies were prepared

according to the American Association of Cereal Chemists (AACC, 10-50D) with slight modifications. For each of the experiment, 200g of the blended flour (CLF – Cereal: Legume: Fruit) was used. Other ingredients included sugar, margarine, skimmed milk powder, baking powder, eggs, vanilla essence and cinnamon

powder were used in the cookie recipe. Sugar was creamed with the margarine to a fluffy consistency using a hand mixer (Geepas Hand Mixer – GM6127). Whole egg and vanilla essence were added to the creamed mixture and whisked to a soft texture. Flour and all the dry ingredients were sieved together and mixed gently with the creamed mixture to obtain a stiff paste. The dough obtained was rolled out on a board and cookie cutters used to shape out the cookies. Cut-out pieces of cookies were placed on a tray lined with parchment paper and baked at 180° C for 15-20 minutes. Cookies were cooled and stored in 500g plastic containers at ambient temperature (30 ± 2°C) for further analysis.

## 2.9 Analysis

### 2.9.1 Physical Analysis of Cookies

Physical parameters of the cookies such as weight, thickness, diameter and the spread ratio (diameter/thickness) were estimated for all the 12 samples of the cookies as described by Kaur et al. (2019). Cookie diameter and thickness were determined using a Vernier calipers while the weights were determined using an electronic weighing balance. The average values obtained for the cookie sample replicates were recorded.

### 2.9.2 Color Analysis of Cookies

Color measurement of the cookie samples was carried out using a Colorimeter (Model NO PCE-CSM 1) fitted with an optical sensor. Samples were analyzed in triplicate and the mean values obtained within 24 hours after baking of the cookie samples. Color measurement was on the basis of CIE  $L^*$ ,  $a^*$ ,  $b^*$  system where the  $L^*$  values measure black to white (0-100),  $a^*$  values

measure the redness when positive while the  $b^*$  values measure the yellowness when positive.

### 2.9.3 Chemical Analysis

Proximate composition of the cookie samples was analyzed according to AOAC (2012) methods. in terms of moisture content (method 925.10) and ash in a muffle furnace at 550°C (method 923.03). Fat was extracted with petroleum ether by Soxhlet (method 922.06C). Protein determination was by the Kjeldahl method (method 920.87), crude fiber (method 920.86) and carbohydrate content (by difference method). Energy value (kcal per 100g) was determined using the Atwar conversion factor as reported by Adeyeye et al. (2017). Samples were ground into fine particles before each of the analysis.

### 2.9.4 In Vitro Protein Digestibility

In Vitro protein digestibility was done according to the method by Afify et al. (2012) with reference to ISO-16634-1 International Standard. One gram of the sample was added to 15ml (0.1M) HCl containing 1.5mg pepsin and incubated for 3 hours at 37°C. 7.5 ml (0.2M) NaOH was then used to neutralize the obtained suspension which was then treated with 4 mg of pancreatin in 7.5 ml (0.2M) phosphate buffer (pH 8.0). Toluene was added to prevent microbial growth and mixture was shaken and incubated for another 24 hours. After the incubation period, 10 ml of 10% TCA was added and centrifuged for 20 minutes to remove the undigested protein and larger peptides from the sample. Kjeldahl method (AOAC, 2012) was used to estimate the protein in the supernatant. Nitrogen in a blank sample was also estimated. Protein digestibility was calculated by the following formula;

$$\text{Protein Digestibility \%} = \frac{\text{Nitrogen in Supernatant} - \text{Nitrogen in Blank}}{\text{Nitrogen in Sample}} \times 100$$

### 2.9.5 Vitamin C Determination

Estimation of Vitamin C in the samples was by the modified method of Barakat et al. (1955) which involved action of *N*-Bromosuccinimide on

ascorbic acid. The procedure involved extraction of vitamin C from a known amount of solid sample with 10% TCA solution to obtain the extract or filtrate. In 5ml of the filtrate, 5ml of 4%

KI solution and some drops of starch solution indicator were added. The mixture was then titrated against 0.1g/liter of N-Bromosuccinimide solution. Starch indicator solution was prepared by mixing 1g of soluble starch in 10ml of hot distilled water which was then cooled and made up to 100ml with saturated NaCl solution. N-Bromosuccinimide solution was prepared by dissolving 10mg of N-Bromosuccinimide in hot distilled water, cooling and topping up the solution to 100ml then stored at 40°C.

### 2.9.6 Vitamin B1 Determination

Vitamin B<sub>1</sub> in the samples was analyzed according to the AOAC method (953.17). 1.5 g

of the sample was added to 100ml of 0.1N HCL and placed in a water bath at 100° C for 30 minutes. The cooled solution was topped up with HCL then filtered and centrifuged to obtain separate layers. Absolute alcohol was mixed with a solution of 5ml of potassium ferric-cyanide in sodium hydroxide. 10ml of toluene was added in the solution and centrifuged. Thiamine standard was also be prepared using the same process. The standard solution and the sample solution were read at 530 nm wavelength using a spectrophotometer.

$$\text{Thiamine (mg/100g)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \frac{\text{Weight of standard (mg)}}{\text{Weight of sample (g)}} \times 100$$

### 2.9.7 Vitamin B2 Determination

Vitamin B<sub>2</sub> was analyzed as per the method illustrated by Adeyeye et al. (2017). 1.5g of each of the cookie sample weighed and mixed with equal ratios of acetic acid and water (50:50) then heated in a 100° C hot water bath for 30 minutes.

The mixture was cooled to 20° C and topped up with a solution of acetic acid mixed with water. Stirring of the mixture was done before being filtered in the dark. A standard riboflavin solution was also be prepared and both solutions will be read using the spectrophotometer according to the AOAC method.

$$\text{Riboflavin (mg/100g)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \frac{\text{Weight of standard (mg)}}{\text{Weight of sample (g)}} \times 100$$

### 2.9.8 Statistical Analysis

Analysis was done in triplicate and all the data expressed as mean ± SD for each of the samples and data collected was subjected to analysis of variance (ANOVA) to determine the significant differences between the treatments. Mean values were compared by Tukey's LSD test (p < 0.05) using GenStat statistics software package version 15.

## 3.0 Results

### 3.1 Physical Properties of the RTE cookies

The physical properties (weight, diameter, thickness and spread ratio) of the cookies are presented in **Table 3.1**. Weight of the cookies made from whole grain sorghum flour decreased significantly (p ≤ 0.05) from 9.96 to 8.26. Weight of cookies from malted sorghum flour ranged from 10.40 g the highest to 8.97g while those from fermented sorghum flour decreased from 11.43g to 9.36g respectively. Spread ratio

ranged from 9.82 (cookies with fermented sorghum flour and 30% green gram flour) to 13.68 (100% sorghum flour cookies which was the control) presenting a decreasing trajectory.

**Table 3.1: Physical Properties of RTE Cookies from Sorghum-Green Gram Blends**

Cookie Samples	Weight (g)	Diameter (mm)	Thickness (mm)	Spread Ratio
CLF 1 - Control	9.96 ± 0.15 <sup>ef</sup>	44.09 ± 0.07 <sup>a</sup>	3.22 ± 0.02 <sup>a</sup>	13.68 ± 0.07 <sup>h</sup>
CLF 2	9.76 ± 0.15 <sup>de</sup>	44.34 ± 0.10 <sup>ab</sup>	3.36 ± 0.01 <sup>b</sup>	13.18 ± 0.03 <sup>g</sup>
CLF 3	8.63 ± 0.15 <sup>b</sup>	44.67 ± 0.07 <sup>cde</sup>	3.56 ± 0.07 <sup>c</sup>	12.47 ± 0.28 <sup>f</sup>
CLF 4	8.26 ± 0.15 <sup>a</sup>	45.48 ± 0.05 <sup>f</sup>	3.89 ± 0.02 <sup>ef</sup>	11.67 ± 0.08 <sup>e</sup>
CLF 5	10.40 ± 0.10 <sup>g</sup>	44.26 ± 0.07 <sup>ab</sup>	3.79 ± 0.01 <sup>d</sup>	11.66 ± 0.06 <sup>e</sup>
CLF 6	9.69 ± 0.08 <sup>cde</sup>	44.34 ± 0.03 <sup>ab</sup>	3.84 ± 0.01 <sup>de</sup>	11.52 ± 0.04 <sup>de</sup>
CLF 7	9.49 ± 0.04 <sup>cd</sup>	44.29 ± 0.24 <sup>ab</sup>	3.92 ± 0.00 <sup>ef</sup>	11.28 ± 0.05 <sup>d</sup>
CLF 8	8.97 ± 0.13 <sup>b</sup>	44.63 ± 0.03 <sup>cd</sup>	3.97 ± 0.00 <sup>f</sup>	11.23 ± 0.02 <sup>d</sup>
CLF 9	11.43 ± 0.09 <sup>h</sup>	44.32 ± 0.02 <sup>ab</sup>	4.13 ± 0.01 <sup>g</sup>	10.72 ± 0.03 <sup>c</sup>
CLF 10	10.53 ± 0.04 <sup>g</sup>	44.44 ± 0.03 <sup>bc</sup>	4.24 ± 0.03 <sup>h</sup>	10.47 ± 0.09 <sup>bc</sup>
CLF 11	10.31 ± 0.06 <sup>fg</sup>	44.82 ± 0.02 <sup>de</sup>	4.35 ± 0.06 <sup>i</sup>	10.30 ± 0.13 <sup>b</sup>
CLF 12	9.36 ± 0.18 <sup>c</sup>	44.093 ± 0.02 <sup>e</sup>	4.57 ± 0.01 <sup>i</sup>	9.82 ± 0.01 <sup>a</sup>

Notes: Values represent mean ± standard deviation of (n=3) replications. Different superscripts in a column are significantly different ( $p \leq 0.05$ ). CLF: Cereal-Legume-Fruit blend. WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5=100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

### 3.2 Color Analysis of the Cookies

Tri-stimulus attributes  $L^*$ ,  $a^*$  and  $b^*$  values were used to express color of the cookie samples which has been presented in **Table 3.2**. Color affects the quality of the final product thus an important criterion to be considered. Significant differences were found in  $L^*$  and  $b^*$  values of the twelve cookie samples. No significant differences ( $p > 0.05$ ) were found in  $a^*$  values however, the values increased in each treatment

corresponding to CLF 1- CLF 4 (from 8.24-8.81), CLF 5 – CLF 8 (from 9.27-9.46) and CLF 9 – CLF 12 (7.27-8.19) respectively. The  $L^*$  (lightness) values decreased significantly ( $p < 0.05$ ) ranging from 52.44-48.60 in cookies with whole grain sorghum flour, 54.91-50.07 in cookies with malted sorghum flour and 50.77-48.24 in cookies with fermented sorghum flour with increasing levels of green gram flour indicating darker products.

**Table 3.2: Color Analysis of the RTE Cookies**

Type of Cookie Sample	L*	a*	b*
CLF 1 (Control -100%)	52.44 ± 0.01 <sup>cd</sup>	8.28 ± 0.01 <sup>a</sup>	8.13 ± 0.97 <sup>ab</sup>
CLF 2	51.12 ± 0.15 <sup>c</sup>	8.72 ± 0.82 <sup>a</sup>	9.66 ± 0.53 <sup>abc</sup>
CLF 3	50.43 ± 0.01 <sup>abc</sup>	8.73 ± 0.04 <sup>a</sup>	11.69 ± 0.75 <sup>c</sup>
CLF 4	48.60 ± 0.01 <sup>ab</sup>	8.81 ± 0.50 <sup>a</sup>	11.91 ± 0.67 <sup>c</sup>
CLF 5	54.91 ± 0.67 <sup>e</sup>	9.27 ± 0.57 <sup>a</sup>	9.59 ± 0.12 <sup>abc</sup>
CLF 6	53.54 ± 0.14 <sup>de</sup>	9.40 ± 1.29 <sup>a</sup>	9.78 ± 0.95 <sup>abc</sup>
CLF 7	51.02 ± 0.78 <sup>c</sup>	9.44 ± 0.21 <sup>a</sup>	10.34 ± 1.88 <sup>bc</sup>
CLF 8	50.07 ± 0.72 <sup>abc</sup>	9.46 ± 0.24 <sup>a</sup>	11.59 ± 1.14 <sup>c</sup>
CLF 9	50.77 ± 0.23 <sup>bc</sup>	7.27 ± 0.55 <sup>a</sup>	6.48 ± 0.50 <sup>a</sup>
CLF 10	48.56 ± 0.14 <sup>ab</sup>	7.41 ± 0.24 <sup>a</sup>	6.56 ± 0.59 <sup>a</sup>
CLF 11	48.36 ± 0.16 <sup>ab</sup>	7.61 ± 0.09 <sup>a</sup>	7.28 ± 0.07 <sup>ab</sup>
CLF 12	48.24 ± 1.64 <sup>a</sup>	8.19 ± 1.15 <sup>a</sup>	8.70 ± 0.20 <sup>abc</sup>

Notes: Values represent mean ± standard deviation of (n=3) replications. Different superscripts in a column vary significantly ( $p \leq 0.05$ ). CLF: Cereal-Legume-Fruit blend. WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5=100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

### 3.3 Effect of green gram flour addition on chemical composition of the cookies

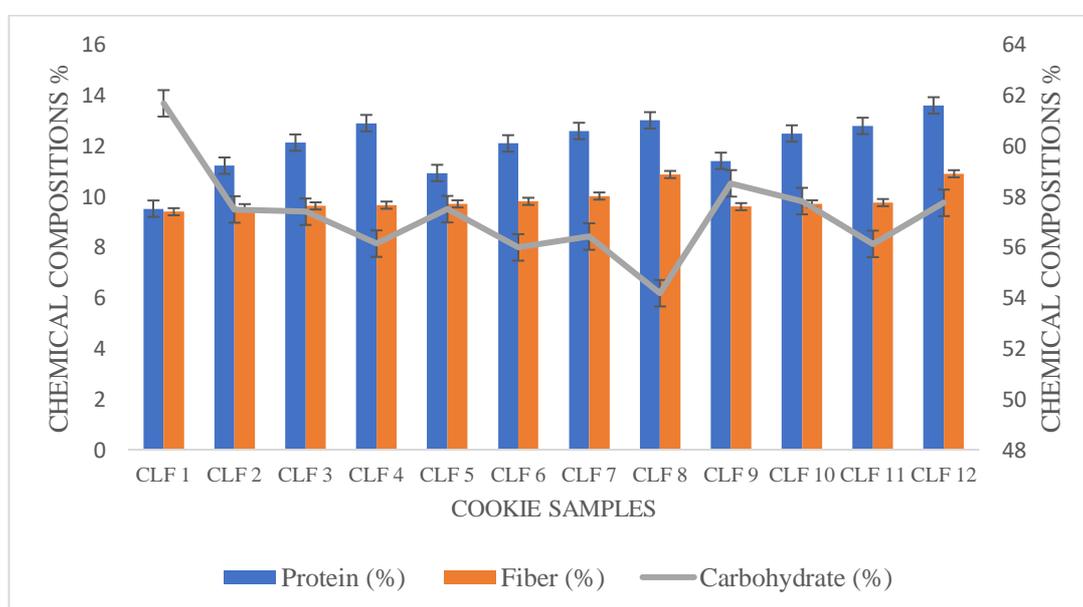
The chemical composition of the different cookie samples produced from the different blends of sorghum flour, green gram flour and dried mango powder is presented in **Table 3.3**. Moisture content of the cookies was less than 10% which is generally low and therefore less likely to cause any adverse effects on the quality attributes of the end products thus prolonging their shelf life. Ash, protein, fat and fiber contents of the cookies increased with increased substitution with green gram flour while carbohydrates decreased significantly from 61.68 to 54.18%. Significant differences ( $p < 0.05$ ) were obtained in the protein content of the cookies which ranged from 9.52 to 13.60 %. Cookies from malted and fermented sorghum

flour registered high protein contents as compared to whole grain sorghum which suggests that malting and fermentation of sorghum grains had a positive effect on the cookies. The ash content though not statistically different ( $p > 0.05$ ), ranged between 1.56% which was the lowest in sample CLF with 100% whole grain sorghum flour to 2.01% in sample CLF 12 with 60% fermented sorghum flour and 30% green gram flour. There were significant differences ( $p < 0.05$ ) in the fiber content which increased with increase in green gram flour and ranged from 9.40 to 10.90 %. According to **Table 3.3**, no significant differences were obtained in the fat content however it increased from 9.89 to 12.77% having the control sample CLF 1 (100% whole grain sorghum flour) registering the lowest fat content.

**Table 3.3: Chemical Composition of the formulated RTE cookies**

Cookie Sample	Moisture(%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	Carbohydrate (%)
CLF 1	7.93±0.68 <sup>a</sup>	1.56±0.40 <sup>a</sup>	9.89±0.80 <sup>a</sup>	9.52±0.11 <sup>a</sup>	9.40±0.00 <sup>a</sup>	61.68±0.36 <sup>b</sup>
CLF 2	7.50±0.60 <sup>a</sup>	1.90±0.10 <sup>ab</sup>	12.26±1.27 <sup>a</sup>	11.22±0.43 <sup>b</sup>	9.56±0.11 <sup>a</sup>	57.49±1.51 <sup>ab</sup>
CLF 3	7.76±0.35 <sup>a</sup>	1.80±0.10 <sup>ab</sup>	11.33±0.72 <sup>a</sup>	12.13±0.32 <sup>d</sup>	9.63±0.05 <sup>a</sup>	57.40±1.04 <sup>ab</sup>
CLF 4	7.90±0.20 <sup>a</sup>	1.88±0.07 <sup>ab</sup>	11.51±1.04 <sup>a</sup>	12.90±0.04 <sup>ef</sup>	9.66±0.05 <sup>ab</sup>	56.14±1.22 <sup>a</sup>
CLF 5	7.76±0.05 <sup>a</sup>	1.91±0.04 <sup>ab</sup>	12.18±1.72 <sup>a</sup>	10.93±0.53 <sup>b</sup>	9.71±0.12 <sup>abc</sup>	57.50±1.36 <sup>ab</sup>
CLF 6	7.40±0.10 <sup>a</sup>	1.92±0.02 <sup>ab</sup>	12.77±2.22 <sup>a</sup>	12.10±0.12 <sup>cd</sup>	9.81±0.02 <sup>abc</sup>	55.99±2.10 <sup>a</sup>
CLF 7	7.63±0.20 <sup>a</sup>	1.88±0.02 <sup>ab</sup>	11.45±0.87 <sup>a</sup>	12.59±0.03 <sup>de</sup>	10.02±0.63 <sup>abc</sup>	56.42±1.63 <sup>a</sup>
CLF 8	7.63±0.21 <sup>a</sup>	1.93±0.05 <sup>ab</sup>	12.38±2.31 <sup>a</sup>	13.01±0.21 <sup>ef</sup>	10.87±1.18 <sup>bc</sup>	54.18±2.48 <sup>a</sup>
CLF 9	7.41±0.17 <sup>a</sup>	1.83±0.05 <sup>ab</sup>	11.22±0.79 <sup>a</sup>	11.41±0.05 <sup>bc</sup>	9.60±0.05 <sup>a</sup>	58.52±0.63 <sup>ab</sup>
CLF10	7.66±0.20 <sup>a</sup>	1.99±0.10 <sup>b</sup>	10.32±0.45 <sup>a</sup>	12.49±0.02 <sup>de</sup>	9.71±0.80 <sup>abc</sup>	57.82±0.79 <sup>ab</sup>
CLF11	7.60±0.10 <sup>a</sup>	1.92±0.06 <sup>ab</sup>	11.79±1.35 <sup>a</sup>	12.79±0.07 <sup>de</sup>	9.76±0.05 <sup>abc</sup>	56.13±1.24 <sup>a</sup>
CLF12	7.60±0.20 <sup>a</sup>	2.01±0.47 <sup>b</sup>	11.16±1.48 <sup>a</sup>	13.60±0.16 <sup>f</sup>	10.90±0.42 <sup>c</sup>	57.75±2.05 <sup>ab</sup>

Notes: Values with the same superscript(s) within the same column are not significantly different at 5% probability level. Values are mean ± standard deviation of triplicate determinations. CLF: Cereal-Legume-Fruit blend. WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder. Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF4=60%WSF:30%GGF:10%DMP, CLF5=100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.



**Figure 3.1:** Effect of green gram flour addition on key chemical compositions of the cookies. Values are means of three replicates (n=3) and error bars indicate standard deviations of the replicates.

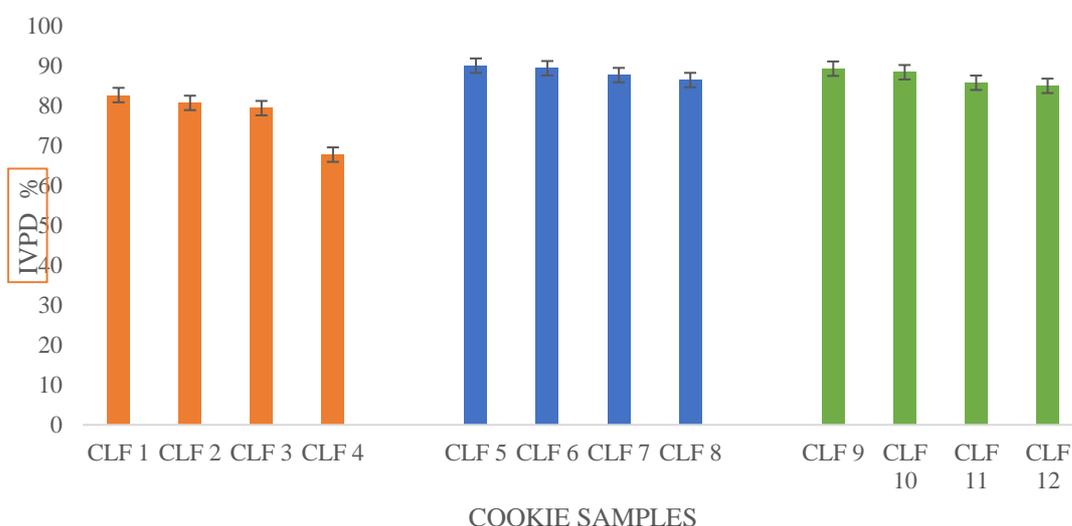
Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

**Figure 3.1** above depicts the trend of the effect of green gram addition on key chemical compositions of the cookies. Protein and fiber increase significantly with addition of the green gram flour. Carbohydrate content decreases with addition of green gram flour. The control sample, CLF 1 with 100% whole grain sorghum flour registered the highest carbohydrate content as compared to the rest of the samples.

### 3.4 Effect of malting and fermentation on In Vitro Protein Digestibility of cookies

The In Vitro Protein Digestibility (IVPD) of cookies from the different blends of cereal, legume and fruit powder flour are shown in

**Figure 3.2.** In vitro protein digestibility of the cookies ranged from  $67.75 \pm 0.010$  to  $90.05 \pm 0.10$  %. In Vitro Protein Digestibility decreased significantly  $p \leq 0.05$  in cookies from whole grain sorghum with the control sample (CLF 1) having 82.68% and CLF 4 having 67.75%. Significant differences ( $p \leq 0.05$ ) were obtained in cookies made from malted sorghum flour and fermented sorghum flour. Cookies from malted sorghum flour had the highest in vitro protein digestibility with values ranging from  $90.05 \pm 0.05\%$  (CLF 5) to  $86.44 \pm 0.00\%$  (CLF 8). Samples CLF 9, CLF 10, CLF 11 and CLF 12 from fermented sorghum flour registered values of  $89.29 \pm 0.29\%$ ,  $88.40 \pm 0.11\%$ ,  $85.76 \pm 0.18\%$  and  $84.99 \pm 0.22$  % respectively.



**Figure 3.2:** In-Vitro Protein Digestibility of cookies formulated. Values are means of three replicates (n=3) and error bars indicate standard deviations of the replicates. Notes: Samples CLF 1 = 100%WSF, CLF2=80%WSF:10%GGF:10%DMP, CLF3=70%WSF:20%GGF:10%DMP, CLF4=60%WSF:30%GGF:10%DMP, CLF5=100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

### 3.5 Vitamin Analysis

**Table 3.4** shows the composition of vitamins mainly B<sub>1</sub> (Thiamine), B<sub>2</sub> (Riboflavin) and Vitamin C (L-Ascorbic Acid) of the cookies. Cookies made from whole grain sorghum (CLF 1, CLF 2, CLF 3, CLF 4) had a thiamine content that ranged from  $0.22 \pm 0.02$  to  $0.41 \pm 0.01$  mg/100g, riboflavin content ranging from  $0.09 \pm 0.00$  to  $0.24 \pm 0.02$  mg/100g while ascorbic

acid ranged from  $13.87 \pm 0.79$  to  $18.99 \pm 0.33$  mg/100g respectively. L-Ascorbic Acid was however not detected in CLF 1 which contained 100% sorghum flour similar to CLF 5 (100% malted sorghum flour) and CLF 9 (100% fermented sorghum flour). Cookies made from malted sorghum flour (CLF 5, CLF 6, CLF 7, CLF 8) had a thiamine content that ranged from  $0.34 \pm 0.00$  to  $0.52 \pm 0.00$  mg/100g, riboflavin

content ranging from 0.32±0.00 to 0.92±0.01 mg/100g while ascorbic acid ranged from 14.56±0.15 to 19.06±0.24 mg/100g. Finally, cookies from fermented sorghum flour (CLF 9, CLF 10, CLF 11, CLF 12) had thiamine contents ranged from 0.38±0.01 to 0.61±0.04 mg/100g, riboflavin from 0.36±0.00 to 1.39±0.04 mg/100g and ascorbic acid from 14.67±0.31 to 19.31±0.94 mg/100g respectively.

**Table 3.4: Vitamin content of the sorghum-green gram RTE cookies**

Type of Cookie Sample	Vitamin B1 (mg/100g)	Vitamin B2 (mg/100g)	Vitamin C (mg/100g)
CLF 1	0.22±0.02 <sup>a</sup>	0.09±0.00 <sup>a</sup>	ND
CLF 2	0.39±0.02 <sup>bc</sup>	0.14±0.00 <sup>ab</sup>	13.87±0.79 <sup>b</sup>
CLF 3	0.40±0.02 <sup>bc</sup>	0.19±0.00 <sup>bc</sup>	17.77±0.14 <sup>cd</sup>
CLF 4	0.41±0.01 <sup>bc</sup>	0.24±0.02 <sup>c</sup>	18.99±0.33 <sup>d</sup>
CLF 5	0.34±0.00 <sup>ab</sup>	0.32±0.00 <sup>d</sup>	ND
CLF 6	0.40±0.02 <sup>bc</sup>	0.49±0.02 <sup>e</sup>	14.56±0.15 <sup>b</sup>
CLF 7	0.46±0.01 <sup>bc</sup>	0.73±0.02 <sup>f</sup>	17.17±0.39 <sup>c</sup>
CLF 8	0.52±0.00 <sup>cd</sup>	0.92±0.01 <sup>g</sup>	19.06±0.24 <sup>d</sup>
CLF 9	0.38±0.01 <sup>bc</sup>	0.36±0.00 <sup>d</sup>	ND
CLF 10	0.46±0.02 <sup>bc</sup>	0.72±0.00 <sup>f</sup>	14.67±0.31 <sup>b</sup>
CLF 11	0.52±0.01 <sup>cd</sup>	0.92±0.01 <sup>g</sup>	17.21±0.33 <sup>c</sup>
CLF 12	0.61±0.02 <sup>d</sup>	1.39±0.04 <sup>h</sup>	19.31±0.94 <sup>d</sup>

Notes: Values are mean ± standard deviation of duplicate determinations. Means on the same column with different sets of superscripts are statistically different ( $p \leq 0.05$ ). Note: ND = Not Detected, WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5=100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

## 4.0 Discussion

### 4.1 Physical Properties of the RTE cookies

The decreasing trend in weight of the cookies was attributed to the increasing fiber content in the cookies as a result of sorghum and green gram flour blend. Diameter and thickness of the cookies increased significantly with each addition of the green gram flour in each treatment. Spread ratio decreased with increasing substitution of green gram flour in the cookies. A similar trend of decreased spread ratio with addition of green gram flour was

reported by Rajiv and Soumya (2015) in biscuits and by Yousaf et al. (2013) in cookies. Decrease in spread ratio and increase in thickness is due to attrition of high protein and bran sources in cookies and biscuits has been reported by different schools of thought (Okpala et al. 2013, Chinma and Gernah 2007, Thongram et al. 2016). Hydrophilic nature of the flour which increased dough viscosity limiting spread in cookies and increasing thickness (Shukla et al. 2016). Increased viscosity is due to the protein network which on heating during the baking process undergoes glass transitioning thus gaining mobility which allows the proteins to

interrelated forming a web (Thongram et al. 2016).

#### **4.2 Color Analysis of the Cookies**

Decrease in  $L^*$  values with increase in green gram flour was as result of the higher protein content in green grams as presented in **Table 3.2**. Previous reports have shown a similar result of decreased  $L^*$  values with increased protein content (Bazaz et al. 2016, Bhise and Kaur 2013). Protein content is correlated negatively with lightness in a baked product due to Maillard reaction which plays a paramount role in color formation due to the interaction of protein and sugar during baking influencing the brown color and organoleptic properties in baked products (Ostermann-Parcel et al. 2017). The redness value ( $a^*$ ) and yellowness values ( $b^*$ ) increased with higher substitution of green gram flour. Increase in similar values with addition of green gram flour was also reported by Rajiv et al. (2013).

#### **4.3 Effect of green gram flour addition on chemical composition of the cookies**

Microbial spoilage has been linked to high moisture which could lead to food spoilage. There were no significant differences ( $p>0.05$ ) in moisture contents of cookies made from whole grain, malted and fermented sorghum flour substituted with green gram flour and fruit powder ranging from 7.40 to 7.93 %. Similar ranges of moisture have been reported other researchers (Onwurafor et al. 2019) in cookies incorporated with unripe plantain and mung bean malt flours. Protein results were similar to those of Rajiv et al. (2012) where protein content in green gram cookies ranged between 10-14%. On average, the Recommended Dietary Allowance (RDA) for protein is 0.8g/kg body weight/day regardless of the age bracket however, infants and children require much more (2-3g/kg body weight/day) for growth and development (Yousaf et al. 2013). Green gram flour can easily be used to increase the protein content of different types of food products (Tsen et al. 2006). Increased ash content was attributed to high mineral content in green grams

such as potassium, phosphorus, magnesium, calcium, sodium and iron (Bazaz et al. 2016). Ash content is an indication of minerals in a food sample. Cereals contain low levels of ash which is in contrast to legumes which are good sources of ash (Dabels et al. 2016). Similar increase in ash and protein contents has been reported in blends with increasing amounts of legumes such as green grams (Rajiv and Soumya 2015), pigeon peas (Okpala et al. 2013), soybeans and kidney beans (Ratnawati et al. 2019). Substitution with green gram flour increased fiber content in cookies. Green grams contain high levels of fiber which makes green grams important in diet and weight management. According to Chavan and Patil (2013), 15g of fiber is provided by a one cup serving of boiled green grams which is over 60% of the daily minimum recommended amount. Increased fiber content due to incorporation of sorghum flour in bread and cookies was reported by Chavan et al. (2016). Dietary fiber plays significant roles in human nutrition with psychological effects such as blood glucose attenuation, blood cholesterol attenuation and laxation and prevention of obesity (Slavin 2005). Increasing trend in fat with addition of green gram flour has also been reported by Bazaz et al. (2016), Hussain and Uddin (2011). Carbohydrates results show that green gram flour is low in carbohydrate and the same is reported by Dabels et al. (2016). Low carbohydrate cookies in this study is paramount to enhanced health for obese and overweight people.

#### **4.4 Effect of malting and fermentation on In Vitro Protein Digestibility of cookies**

**Figure 3.1** shows fermentation and malting processes improved the IVPD of the cookies as compared to cookies made from whole grain sorghum flour despite substitution with green gram flour. Similar improvements in IVPD was observed after fermentation of sorghum flour during processing of injera (Mohammed et al. 2011). Increase in in vitro protein digestibility

after germination of sorghum varieties was also reported by Afify et al. (2012). Low protein digestibility in sorghum is due to anti-nutrients which inhibit protein digestion and resistance of the seed storage protein known as kafirins to protease digestion. Germination of sorghum causes activation of intrinsic proteases, phytases, amylases as well as fiber-degrading enzymes leading to increased nutrient digestibility which increases in vitro protein digestibility (Correia et al. 2010). Enzymatic activity triggered during malting leads to breakdown of proteins, lipids and carbohydrates into simpler forms. Fermentation eliminates antinutrients improving functional, physicochemical and nutritional properties of sorghum flour (Rahayu et al. 2019). Cookies with highest protein content had the least in vitro protein digestibility while those with the least protein had high protein digestibility. High protein content therefore does not necessarily imply high protein digestibility as reported in various studies (Kiin-Kabari and Giambi 2015). Addition of green gram flour decreased the protein digestibility due to various antinutrients such as phytic acid and tannins as explained by Mubarak 2005. Similar decreasing trend in in vitro protein digestibility has also been reported by Bazaz et al. (2016) in hypoallergic complementary food from rice incorporated with sprouted green gram flour.

#### 4.5 Vitamin Analysis

Thiamine in green grams ranges between 0.12-0.7 mg/100g with an average of 0.5mg/100g and a riboflavin average of 0.3mg/100g ranging between 0.23-0.47 mg/100g (Dahiya et al. 2015). Green gram substitution led to increased L-Ascorbic acid content which ranged between  $13.87 \pm 0.79$  –  $19.31 \pm 0.94$  mg/100g as represented in **Table 3.4**. According to Dahiya et al. (2015), ascorbic acid in green grams has a maximum of 10 mg/100g with an average of 3.1 mg/100g. Samples CLF 1, 5 and 9 did not record any trace of ascorbic acid since sorghum has been reported to contain no ascorbic acid. The high L-Ascorbic Acid contents could also be

attributed to the enrichment of the cookies with dried mango powder. Mango fruit (*Mangifera indica*) is rich in L-Ascorbic Acid which ranges from 7.8 to 172 mg/100g (Caparino et al. 2017). Fermentation has been reported to influence the production of vitamin B-complex by different schools of thought (Obadina et al. 2013). Results of fermented cookies are almost similar to those of Adeyeye et al. (2017). General functions of thiamine are in assisting the body to produce energy as well as protect the mucous membrane and nervous system while riboflavin maintains a healthy digestive system, healthy red blood cells, skin and vision (Duru et al. 2012, Ndung'u et al. 2015). Vitamin C contents are however lower than those reported by Nilugin et al. (2015) in cookies substituted with 25% mango flour. High amounts of sugar, considerable amounts of vitamin C and provitamin A in mango makes it a fruit of high commercial value with added health benefits such as prevention of scurvy (Fenech et al. 2019). Reduces risks of cardiovascular diseases and certain cancers has been linked to increased consumption of mango fruit (Adepoju and Osunde 2017).

#### 5.0 Conclusion

Fermentation and malting of sorghum grains improved the nutrient composition of the cookies in addition of the green gram flour which is packed with nutrients. Cookies with higher levels of green gram flour coupled with either fermented or malted sorghum flour resulted in an increase in protein and vitamin contents. *In Vitro* Protein Digestibility was highly influenced by fermentation and malting which improved the results by a greater percentage as compared to samples from whole grain sorghum flour. Combination of a cereal-legume -fruit flour blend will increase utilization of neglected crops as well as ensure healthy quality snacks such as cookies which due to the abundance of nutrients can help in countering protein energy malnutrition especially in developing countries.

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### Conflict of Interest

The authors declare no conflict of interest.

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