



Phytochemical, Scavenging Properties and Glycemic Index of Soy-Enriched Maize-Based Gruel Fortified with Moringa Leaves and Wonderful Kola

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ABA and OSI designed the study, author JHG performed the statistical analysis, author ABA wrote the protocol, and wrote the first draft of the manuscript. Authors ABA and OSI managed the analyses of the study. Authors ABA and JHG managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2018/40375

Editor(s):

(1) Aneta Popova, Chief Assistant Professor, Department of Catering and Tourism, University of Food Technologies, Bulgaria.

Reviewers:

- (1) Eliene Penha Rodrigues Pereira, Universidade Federal Fluminense, Brazil.
(2) Oluwatoyin Ajoke Oladeji, Southern Nigeria Institute of Innovative Technology, Nigeria.
(3) Harimalala Andriambelo Nirina, University of Antananarivo, Madagascar.
Complete Peer review History: <http://prh.sdiarticle3.com/review-history/24560>

Received 26th February 2018

Accepted 30th April 2018

Published 11th May 2018

Original Research Article

ABSTRACT

In this study, defatted soybean cake, *Moringa oleifera* leaves and wonderful kola combination were used to raise the nutritional value and scavenging properties of maize-based formulated diets. After blanching and fermentation process, maize (popcorn), moringa leaves, wonderful kola seeds, defatted soybean cake were milled into flour and blended to obtain six samples (R1, R2, B1, B2, F1, F2). Samples were analyzed for antinutrients, phytochemicals, scavenging properties and glycemic index using standard methods. Both processing methods reduced antinutrient levels; particularly for phytic acid. Of the methods used, fermentation is the most effective in increasing the protein content of the formulated diets, it also had the highest scavenging ability and the lowest glycemic index. For DPPH (1,1 diphenyl-2-picrylhydrazyl) assay, R1, B1, F1 had high scavenging abilities with F1 having the highest. Albino rats fed with 1 g of sample F1 showed a great decrease in blood glucose

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level below 5 mmol/l when compared with other samples and reference sample (glucose). This shows the importance of a healthy diet and the effectiveness of *Moringa oleifera* leaves in the prevention and management of diabetes.

Keywords: *Moringa oleifera*; wonderful kola; maize; phytochemical; glycemic index.

1. INTRODUCTION

Cereals are widely utilized as food in Nigeria as well as in other African countries than in developed world; according to Makinde et al. [1]. Maize is one of the most prominent of these cereals and it constitutes over 60% of the cereals consumed. Attempts are currently being made in developing world where maize is a major staple to supplement it with certain conventional legumes as soybean that contains adequate amounts of limiting amino acids. However, maize protein is deficient in lysine and tryptophan but has fair amount of sulphur-containing amino acids (methionine and cystine) while legume protein are relatively rich source of lysine and tryptophan but low in sulphur amino acids thereby making it an excellent raw material to improve the nutritional quality of maize-based products [2]. Popcorn (*Zea mays var everta*) is a variety of maize that is densely starchy and when processed it contains majorly carbohydrate and lacks most of the other nutrients [3]. Soybean (*Glycine max*) is a species of legume that is considered to be a source of complete protein and the meal is the material remaining after solvent extraction of oil from soybean flakes, with 50% soy protein content. Rubin et al. [4] described *Moringa oleifera* (drum tree), as a medicinal plant that belongs to *Moringaceae* family with 14 species. The leaf, seeds and flowers all have good nutritional and therapeutic values. Study by Tete-Benissan et al. [5] has shown that the leaves were used to prevent or treat nutritional related diseases. *Moringa oleifera* has anti-inflammatory [6], anti-cancer [7], thyroid status regulatory potentials [8] and researches reported its hypoglycemic potential [9]. Ezekiel [10] reported that *Bucchozia coriacea*, locally known as 'wonderful kola' belonging to the family Capparaceae is traditionally macerated in water or local gin as a cure for diabetes. The leaves and seed have been reported to have anti-helminthic activity [11] as well as antimicrobial properties [12]. The American Diabetes Association [13] defined diabetes as "a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both". This may also be associated with sedentary lifestyle and dietary changes. The

International Diabetes Federation estimated about 415 million adults worldwide who are affected by diabetes and this figure is expected to reach 642 million by 2040 [14] while type 2 diabetes accounts for more than 90% of all the diabetic cases. It has become a major cause of death in people under the age of 60. Diets rich in whole grains, fruits, vegetables, legumes, nuts and lower in refined grains, red/processed meats and sugar sweetened beverages has shown reduced diabetes risk, improved glycemic control in patients living with diabetes. However, Dietary modification is the simplest and cheapest form of diabetes treatment and is a clinically recommended primary therapy in type 2 diabetes [15]. Due to the high contents of phytochemicals and micronutrients in moringa leaf and wonderful kola, their use as fortificants in maize-based meals is expected to raise the nutritional value and scavenging properties of such foods. The objectives of this study is therefore to determine the proximate composition, phytochemical composition, scavenging properties and investigate the glycemic index of the formulation using Wistar rat models.

2. MATERIALS AND METHODS

2.1 Sources of Materials

Defatted Soybean (*Glycine max*) cake was purchased from Jof Ideal Family Farms, Owo. Maize – Popcorn (*Zea mays everta*) was purchased from Oba market in Akure. *Moringa oleifera* leaves were plucked from moringa tree within FUTA community. Wonderful Kola (*Bulcchozia. coriacea*) was purchased from Oje market in Ibadan.

2.2 Processing of Defatted Soybean Flour

The defatted soybean cake was milled using electric blender, sieved with 250mm sieve to obtain the defatted soybean flour.

2.3 Processing of Fermented Maize Flour

Maize seeds were sorted, soaked in water for 3 days, washed with portable water and wet milled

using electric blender to get slurry that was soaked for another 3 days to ferment. After 3 days it was decanted, then oven dried for 3 days in hot air oven at 60°C. Then it was dry milled and sieved using 250 mm sieve to obtain the fermented maize flour.

2.4 Processing of Raw, Blanched and Fermented Moringa Leaves Flour

The fresh moringa leaves were sorted; shade dried at room temperature for 2 weeks, then milled using electric blender and was sieved using 250 mm sieve to obtain raw moringa leaves flour.

The fresh leaves were sorted; steam blanched for 30 minutes and then oven dried at 40 °C for 72 h; then milled and sieved to obtain blanched moringa leaves flour.

The fresh leaves were sorted; steam blanched for 30 minutes and immediately the blanched leaves were tightly wrapped in plantain leaves and allowed to ferment for 72 h; after which it was untied. Then the steam blanched, fermented leaves were oven dried at 40°C for 72h and milled, sieved to obtain fermented moringa leaves flour.

2.5 Processing of Raw, Blanched, Fermented Wonderful Kola Seeds Flour

The raw seeds were sorted, dehulled, peeled, cut and cleaned. Then oven dried at 60°C for 72 h, milled and sieved to obtain raw wonderful kola seed flour.

Cleaning was followed by blanching for 30 minutes before decanting. Then oven drying at 60°C for 72 h. Blanched seeds were milled using electrical blender and sieved to obtain blanched wonderful kola seed flour.

After cleaning, the seeds were blanched, decanted and allowed to ferment for 3 days (same as in 2.4), then it was oven dried at 60°C for 72 h, followed by milling and sieving to obtain fermented wonderful kola seed flour.

2.6 Formulation of Blends

The popcorn, defatted soybean cake, moringa leaves (raw, blanched, fermented) and wonderful kola seeds (raw, blanched, fermented) flours

were mixed at different proportions to give 6 samples as; as R1 (60% Popcorn, 20% Deffated soybean cake, 20% Raw *Moringa* leaves flour), R2 (60% Popcorn, 20% Deffated soybean cake, 20% Raw wonderful kola seed flour), B1 (60% Popcorn, 20% Defatted soybean cake, 20% Blanched *Moringa* leaves flour), B2 (60% Popcorn, 20% Defatted soybean cake, 20% Blanched wonderful kola seed flour), F1 (60% Popcorn, 20% Defatted soybean cake, 20% Fermented *Moringa* leaves flour), F2 (60% Popcorn, 20% Defatted soybean cake, 20% Fermented wonderful kola seed flour). Electric blender was used to mix the samples to achieve uniform blending.

2.7 Proximate Analysis of Formulated Flour Samples

The moisture content, Ash, Crude fat, Crude fiber, Protein and Carbohydrate were determined by method of AOAC, 2005 [16]. Moisture content was determined by drying 5g of sample using a drying oven at 105 °C for 5 h until a constant weight was attained. Crude protein content was determined by the Kjeldahl method with a 6.25 conversion factor. Fat content was determined by gravimetric analysis after soxhlet extraction. Crude ash was estimated by incineration in a muffle furnace (Felisa, 360 D) at 550°C for 30 minutes.

2.8 Phytochemical Properties of Formulated Flour Samples

Total phenol: The total phenol content was determined by the method of Singleton et al [17]. About 0.2 ml of the extract was mixed with 2.5 ml of 10% Folin ciocalteau's reagent and 2 ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45°C for 40 min, and the absorbance was measured at 700 nm in the spectrophotometer, gallic acid was used as standard phenol.

Total flavonoid: The total flavonoid content was determined using a colorimeter assay developed by Bao et al [18]. About 0.2 ml of the extract was added to 0.3 ml of 5% NaNO₃ at zero time. After 5 min, 0.6 ml of 10% AlCl₃ was added and after 6 min, 2 ml of 1M NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

Alkaloid: Alkaloid was determined using the method of Harbone [19]. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid and ethanol was added and allowed to stand for 4 min. Then it was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute Ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

$$\% \text{ Alkaloid} = \frac{w3 - w2}{w1} \times 100$$

2.9 Anti-nutritional Composition of Formulated Flour Samples

Phytate: Phytate was determined using the method of AOAC, 2005 [16]. Phytate was extracted using dilute HCl and extract was mixed with Na₂EDTA – NaOH solution, and placed in an ion-exchange column. The extracted phytate is diluted with 0.7ml NaCl solution and wet-digested with H₂SO₄/HNO₃ mixture to release phosphate, which was measured colorimetrically after reacting with ammonium molybdate solution. The amount of phytate in original sample is obtained as hexaphosphate equivalent. Phytate concentration in the diet samples was extrapolated from the generated standard curve, and expressed as mg/100 g sample.

Tannin: Tannin was determined by the modified vanillin-HCl methods [20]. A 2 g sample was extracted with 50 ml 99.9% methanol for 20 minutes at room temperature with constant agitation. After centrifugation for 10 min. at 653 rpm, 5 ml of vanillin-HCl (2% vanilli and 1% HCl) reagent were added to 1 ml aliquots, and the colour developed after 20 min. at room temperature was read at 500 nm. Correction for interference light natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction, but without vanillin reagent. A standard curve was prepared using catechin (Sigma Chemical, St. Louis, MO) after correcting for blank, and tannin concentration was expressed in g/100 g.

Oxalate: Total oxalate was determined using the method of Burns [16]. Oxalate was precipitated as insoluble calcium oxalate, which was collected by centrifuging. The precipitate was dissolved in an excess of hot dilute H₂SO₄ and the oxalate

titrated (in hot) with standardized KMnO₄. The volume of KMnO₄ used to titrate the hot solution of each sample was used to calculate the oxalate content of each sample as follows:

$$\text{Mg per 100g sample} = \frac{(\text{ml of } 0.01N \text{ KMnO}_4 \times 1350)}{\text{weight of sample}}$$

$$\begin{aligned} & \text{Where } 1350 \\ & = 0.45 \left(\frac{\text{mg oxalic acid equivalent to 1ml } 0.01N}{\text{KMnO}_4} \right) \\ & \times \left[\left(\frac{30}{20} \right) \times \left(\frac{50}{25} \right) \text{ dilution factors} \right] \\ & \times 100 \text{ (to convert to 100g sample)} \end{aligned}$$

Trypsin inhibition activity (TIA): The trypsin inhibition activity was determined by method of Kakade et al. [21]. 1g each of the sample was extracted continuously at ambient temperature for 3 hours with 50mL, 10 mM NaOH using a mechanical shaker (GallenKamp orbital shaker Surrey, UK). The pH of the resulting slurry was adjusted to 9.4-9.6 with 1 M NaOH. After extraction, the suspension was shaken and diluted with distilled water such that 1 cm³ of the extract produced trypsin inhibition of 40-60% at 37°C. The respective dilutions will be noted. Consequently, TIA was calculated in terms of mg pure trypsin (Sigma type III, lot 20H0868)

$$\text{TIA} = \frac{2.632DA}{S \text{ mg pure trypsin inhibited g} - 1 \text{ sample}}$$

Where D is the dilution factor, A is the change in absorbance at 410 nm due to trypsin inhibition per cm³ diluted sample extract and S is the weight of the sample.

2.10 Free Radicals Scavenging Ability

DPPH scavenging ability: DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging ability was determined using Gyamfi et al [22] method. 1 ml of the extract was mixed with 1 ml of 0.4 mM methanolic solution. The mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

ABTS scavenging ability: The ABTS 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) scavenging ability of the food samples was determined using the method described by Re et al [23].

2.11 Glycemic Index Determination Using Animal Studies

Twenty one albino rats of wistar strain (150-160 g body weights) were housed in groups of three

in suspended mesh bottom and front stainless steel hanging cages of 25X22X20 cm (fecal collection trays underneath) in a controlled condition, between 20 – 25°C. Deionized distilled water was offered *ad libitum*. After a 12-hour overnight fast, the animals were fed with 1g each of the test diets, which was totally consumed within 30 min. Blood glucose was determined after 1,2,3,4 hour. The same method was performed with the control group giving 1g standard glucose dissolved in distilled water. Blood glucose level was determined by using Accu check[®] glucometer kit. Blood samples were taken from the tail vein after the meal to measure blood glucose levels.

2.12 Statistical Analysis

Statistical Package for Social Sciences (SPSS) software version 16.0 was used to analyze data. The mean and standard deviation of the analysis were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means using Duncan Multiple Range Test at $P < 0.05$.

3. RESULTS

3.1 Proximate Analysis

Table 1 shows the result of the effect of blanching and fermentation on the proximate composition of the formulated food samples. There was no significant difference ($P < 0.05$) between the moisture content of the raw and fermented samples. This result showed that all samples were within normal moisture content range. It was observed that the blanched samples had lower moisture content than raw and fermented samples. Fiber content ranged

from 4.51 to 2.79 g/100g with sample R1 and F2 having the highest and lowest values respectively. The fat content of each sample was significantly different ($P < 0.05$) with values ranging from 13.26 to 10.26 g/100g for B1 and F2 respectively. There was significant difference ($P < 0.05$) between the protein content of raw, blanched and fermented samples. The values ranged from 30.06 to 25.34 g/100g with sample F1 having the highest protein content and sample B2 having the lowest. When compared with the raw samples, it was observed that protein content decreased in the blanched samples while the values increased in the fermented samples. The carbohydrate content ranged from 57.78 to 50.3 g/100g for samples R1 and B2. Sample B2 had the highest value while sample R1 had the lowest value. Generally, it was observed that both blanching and fermentation increased the carbohydrate content though the values greatly increased in blanched samples and slightly increased in the fermented samples.

3.2 Phytochemical and Antinutrient Screening

Table 2 shows that the food samples contain phytochemicals and antinutrients. The antinutrient values were reduced to safe level by the processing treatments. Tannin content ranged from 9.28 mg/100g (R2) to 5.27 mg/100 g (B2), Oxalate values ranged from 0.36 to 0.20 mg/100g in R1 and F2 respectively. Phytic acid ranged from 34.61 mg/100g in sample R2 to 17.3 mg/100g in B2. Saponin ranged from 4.00 to 0.73 mg/100g in samples R2 and B2 respectively. Generally, it was observed that antinutrient contents were lower in both blanched and fermented samples; except for alkaloid whose values were high.

Table 1. Proximate composition of formulated flour samples (mg/100g)

% Comp.	B1	F1	R2	F2	B2	R1
Moisture	6.5 ^b ±0.18	7.17 ^a ±0.04	7.45 ^a ±0.19	7.41 ^a ±0.04	6.2 ^b ±0.16	7.08 ^a ±0.02
Crude fiber	3.43 ^b ±0.38	2.83 ^b ±0.05	3.26 ^b ±0.32	2.79 ^b ±0.07	3.02 ^b ±0.02	4.51 ^a ±0.15
Crude fat	13.26 ^a ±0.41	11.23 ^{bcd} ±0.51	11.56 ^c ±0.10	10.26 ^d ±0.41	10.40 ^{cd} ±0.25	11.72 ^b ±0.14
Ash	3.53 ^b ±0.12	3.22 ^b ±0.1	3.26 ^b ±0.32	3.25 ^b ±0.14	3.48 ^b ±0.08	4.51 ^a ±0.15
Protein	26.01 ^{cd} ±0.05	30.06 ^a ±0.01	27.85 ^{bc} ±0.54	29.16 ^{ab} ±0.95	25.34 ^d ±0.73	27.37 ^{bc} ±0.23
Carbohydrate	53.78 ^b ±0.95	52.67 ^{bc} ±0.36	54.09 ^b ±0.19	54.56 ^b ±1.30	57.78 ^a ±0.91	50.3 ^c ±0.5
Energy(Kcal)	438.48 ^a ±0.09	431.98 ^{ab} ±3.11	431.72 ^b ±2.05	427.15 ^b ±2.32	425.98 ^a ±1.44	416.06 ^c ±0.14

Mean values with the same superscript in a row are not significantly different ($P > 0.05$)

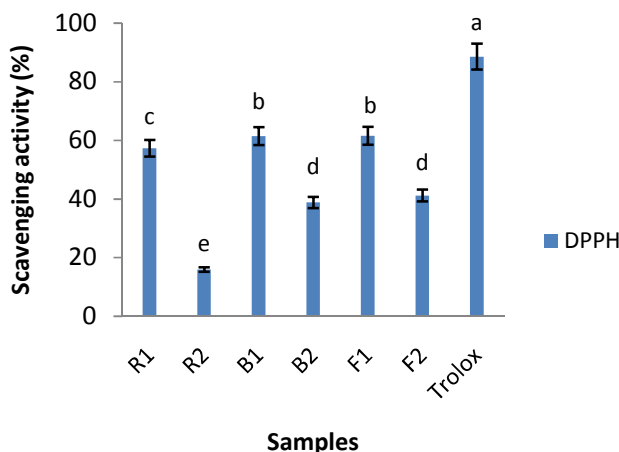
Note: R1(60% Popcorn flour, 20% Defatted soybean cake, 20% Raw moringa leaves flour), R2(60% Popcorn flour, 20% Defatted soybean cake, 20% Raw wonderful kola flour), B1(60% Popcorn flour, 20% Defatted soybean cake, 20% blanched moringa leaves flour), B2(60% Popcorn flour, 20% Defatted soybean cake, 20% Blanched wonderful kola flour), F1 (60% Popcorn flour, 20% Defatted soybean cake, 20% Fermented moringa leaves flour), F2(60% Popcorn flour, 20% Defatted soybean cake, 20% Blanched wonderful kola flour).

Table 2. Phytochemical and anti nutrient composition of formulated food samples (mg/100g)

	R1	R2	B1	B2	F1	F2
Tannin	7.92 ^b ±0.03	9.28 ^a ±0.27	7.14 ^e ±0.00	5.27 ^d ±0.19	7.22 ^c ±0.24	7.56 ^{bc} ±0.01
Phenol	3.95 ^a ±0.000	2.71 ^d ±0.012	3.70 ^c ±0.000	2.36 ^f ±0.014	3.74 ^b ±0.007	2.49 ^e ±0.002
Phytic	32.55 ^b ±0.412	34.61 ^a ±0.824	19.78 ^c ±0.001	17.30 ^d ±0.001	31.31 ^b ±0.000	20.6 ^c ±0.000
Saponin	2.27 ^b ±0.091	4.00 ^a ±0.182	1.55 ^d ±0.091	0.73 ^e ±0.001	2.55 ^b ±0.001	1.91 ^c ±0.091
Flavonoid	1.27 ^{bc} ±0.017	8.98 ^a ±0.243	0.97 ^c ±0.001	0.97 ^c ±0.001	1.94 ^b ±0.486	0.95 ^c ±0.021
Oxalate	0.36 ^a ±0.002	0.27 ^a ±0.001	0.27 ^a ±0.002	0.36 ^a ±0.001	1.98 ^a ±1.620	0.36 ^a ±0.001
Trypsin	0.30 ^e ±0.001	0.45 ^b ±0.005	0.33 ^d ±0.005	0.48 ^a ±0.005	0.29 ^f ±0.000	0.40 ^c ±0.001
Alkaloid	0.12 ^a ±0.646	0.10 ^b ±0.646	0.44 ^c ±1.000	2.77 ^d ±1.000	0.76 ^e ±1.000	4.65 ^e ±1.000

Mean values with the same superscript in a row are not significantly different ($P>0.05$)

Note: R1(60% Popcorn flour, 20% Defatted soybean cake, 20% Raw moringa leaves flour), R2(60% Popcorn flour, 20% Defatted soybean cake, 20% Raw wonderful kola flour),B1(60% Popcorn flour, 20% Defatted soybean cake, 20% blanched moringa leaves flour),B2(60% Popcorn flour, 20% Defatted soybean cake, 20% Blanched wonderful kola flour), F1 (60% Popcorn flour, 20% Defatted soybean cake, 20% Fermented moringa leaves flour), F2(60% Popcorn flour, 20% Defatted soybean cake, 20% Blanched wonderful kola flour)

**Fig. 1. The DPPH scavenging activity of formulated food samples in %**

3.3 Free Radical Scavenging Activity

Fig. 1 and 2 shows the antioxidant property of the formulated food samples. For DPPH assay, the result shows that sample R1, B1, F1 fortified with raw, blanched and fermented moringa leaves had the highest scavenging ability as compared with Trolox used as standard while sample R2 had the weakest scavenging ability. For the ABTS assay, samples R1 and F1 had the highest scavenging ability while samples B2, F2 had very weak scavenging ability.

3.4 Glycemic Index

This graph shows how the blood glucose level of the Albino rats responded to the formulated diet. Glucose was used as reference standard. It was observed that oral glucose caused a rapid increase in blood glucose level of the rats which

peaked at 1 h and then decreased steadily after 1 h. Each of the formulated diets showed an initial gradual increase in blood glucose level at 1 h after which a steady decrease was observed in the glycemic response of the rats in samples B1 and F1. Sample F1 decreased the value below 5 mg/dl after 3 hour while sample R2 showed a wide range of blood glucose concentration after 3 h.

4. DISCUSSION

4.1 Proximate

The result of the proximate analysis showed that all the samples were within normal moisture content range and it was observed that the blanched samples had lower moisture content than both raw and fermented samples. This observation implies that the blanched samples

may have longer shelf life than the other samples as it will not be as liable to microbial spoilage. The values were lower when compared with values obtained by Ijarotimi & Oluwalana [24]. The fiber content was highest in raw samples and lower in other samples. The fermented samples have the lowest value. This corresponds with the report of Oluwaseun et al. [25] that fiber content tends to decrease during fermentation and this could be as a result of the breaking down of carbohydrate, crude fiber and other complex organic substances in the fermented substrate by fermenting organism during fermentation; thereby reducing the fiber content

of such food. Odenigbo [26] reported that fiber is important in glycemic control and improved morbidity of diabetic patients. It was observed that fat content was highest in blanched sample (B1) which is in agreement with the report of Sengev [27] that fat content increased with increasing moringa supplementation while sample F2 had the lowest value. According to Gordon & Kessel [28], low fat foods are said to reduce the level of cholesterol and obesity. For the ash content, sample R1 was significantly different from other samples. This could be attributed to the leaching of soluble mineral element into water during

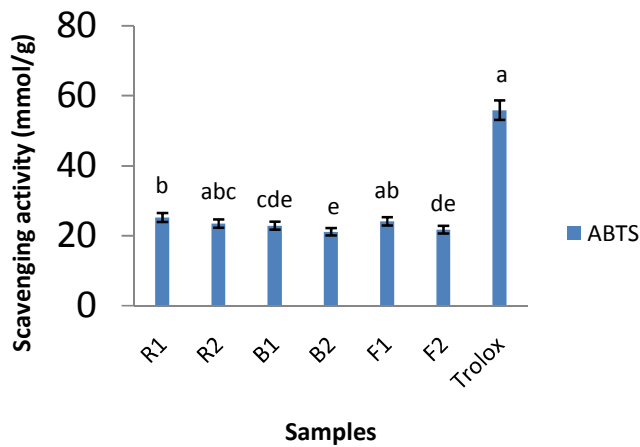


Fig. 2. The ABTS scavenging activity of formulated food samples (mmol/g)

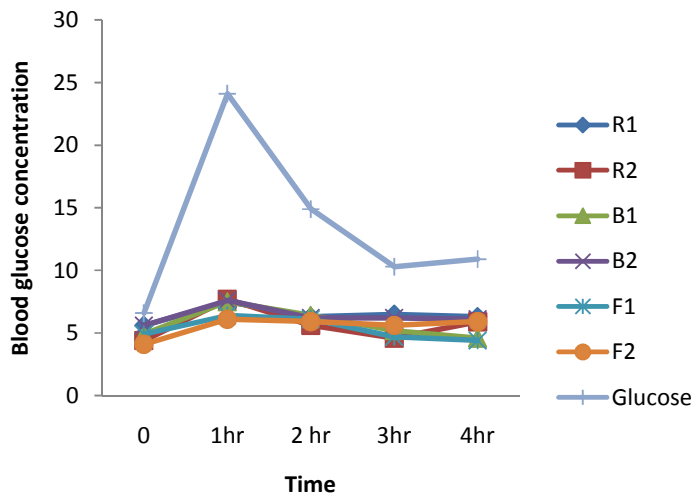


Fig. 3. Effect of formulated food samples on the blood glucose level of the rats in mmol/l

blanching and the usage of these minerals by interest microorganisms for metabolic activities during fermentation. This agrees with the report of Michodjehoun et al. [29] that there was a decrease in ash content during fermentation of "Gowe" a traditional food made from sorghum, maize. Protein content was highest in fermented samples with sample F1-30.06% having the highest value and lowest values were observed in blanched samples. This increase in protein content could be attributed to increase in microbial mass during fermentation, causing extensive hydrolysis of protein molecules to amino acid and other simple peptides or as a result of the enzymatic hydrolysis of some protease inhibitors during fermentation. This increase corresponds with the observation of Michodjehoun [29] on increase in protein content from 7.9 to 10 % during fermentation of millet. This could also be attributed to the rich protein and micronutrient content of *Moringa oleifera* leaf as reported by Edward [30]. Carbohydrate content was highest in blanched sample (B2) containing blanched Wonderful kola flour and lowest in sample R1. The high value observed in sample B2 could be attributed to the high carbohydrate content of *Bucchozia coriacea* seed which is about 77.18% as reported by Amechi [31]. It was also observed that both processing methods increased the energy value in samples B1 and F1. The values reduced in samples B2 and F2, although the values were lower than the values obtained by Igile [32]. The US Department of Agriculture, 1995 recommends that low energy diets provide substantial weight loss and rapid improvement in glycemia in individuals with type 2 diabetes.

4.2 Phytochemical and Antinutrients

Phytochemicals, especially phenolics are known to be major bioactive compounds for health benefits. Therefore, it was observed from the result that both blanching and fermentation improved the nutritional quality of the flour samples and also reduced the anti-nutritional factors in the food samples to safe levels. Numerous studies have indicated that these anti-nutrients can be eliminated or reduced significantly by processing techniques such as thermal heating, milling, soaking, fermentation, germination, cooking and protein extraction [33]. This can also be attributed to the report of Spiriya [34] that fermentation reduces the level of anti-nutrients such as phytic acid, tannin in food leading to bioavailability of minerals such as protein, iron. El-Adawy [35] reported that cooking

treatment such as blanching causes significant decrease in anti-nutritional qualities of these compounds thereby permitting the body to absorb nutrient from them. According to Sotelo [36], tannins are water soluble phenolic compounds which precipitate protein from aqueous solution. They bind to protein making them bio-unavailable. Tannin was observed in high amount in raw samples R1, R2 while the values reduced in the fermented and blanched samples. Pinent et al. [37] described tannins as anti-hyperglycemic agents in diabetic rats. The reduction in blood glucose levels caused by phenolic compounds have been attributed to actions such as reduction in the absorption of nutrients (e.g catechins which inhibits intestinal glucose absorption) [38], reduction in food intake [39], induction of β cell regeneration [40] and a direct action on adipose cells that enhances insulin activity [41]. Studies show that oxalate in large amount bind with calcium forming oxalate which is insoluble and not absorbed by the body [42] and they are considered as poisonous at high concentration but harmless when present in small amount [43]. Phytate content of the raw samples were very high while both blanching and fermentation reduced the value. Saponins are known to inhibit growth of cancer cells, lower cholesterol levels, boost immunity and energy and also acts as a natural antibiotic [44].

4.3 Free Radical Scavenging Ability

Samples B1 and F1 was significantly different from other samples and they had the highest values. This implies that sample B1 and F1 had higher scavenging property. This could be attributed to the high scavenging ability of moringa leaf as reported by Pakade et al. [45]. Moringa plant contain important minerals, good source of protein, vitamins and various phenolics such as zeatin, quercetin and about 46 antioxidants which help cells to neutralize free radicals [46]. Choi et al. [47] investigated the influence of heat treatment on the antioxidant activity and polyphenolic compounds in shiitake mushroom (*Lentinus edodes*) and reported that antioxidant activity increases with heating temperature. This happens because the heat treatment liberates phenolic compounds and thus increasing the amount of active compounds.

4.4 Glycemic Index

This result shows that oral glucose used as reference standard caused a rapid increase in blood glucose response of the rats but started

decreasing from 1hr which could indicate complete absorption of glucose within a short time. However, the food samples showed an initial gradual increase in glycemic response of the rats which later decreased. It was observed that rats fed with sample F1 showed a great decrease in blood glucose level below 5mmol/l after 3hr and this corresponds with the recommendation of American Diabetes Association (ADA) that blood glucose level < 7mmol/l is normal. This result contradicts the report of Ihediohanma [48] that blood glucose response to a food material increases with increase in fermentation time.

5. CONCLUSION

The present study established the chemical composition, phytochemical and scavenging properties of formulated soy bean enriched maize based diet fortified with moringa leaves and wonderful kola flour. Also, the result showed that both fermentation and blanching improved the nutritional composition of the formulated diet particularly sample fortified with fermented moringa leaf flour (F1); in terms of increase in protein and decrease in fat content, reduced antinutrient content, high free radical scavenging ability and a great reduction in the blood sugar level of the rats.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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