

Physiochemical, Anti-nutrient and *in-vitro* Protein Digestibility of Biscuits Produced from Wheat, African Walnut and *Moringa* Seed Flour Blends

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Authors' contributions

This work was carried out in collaboration among all authors. Author VCW is a PhD student while the others are his supervisors. Author VCW managed the literature searches of the study, performed the work and carried out statistical analysis. Authors SYG and DBKK authors designed the study. Authors DBKK and OMA authors wrote the protocol and first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Biscuits were produced from wheat, African walnut and *Moringa* seed flour blends and chemical, physical, *in-vitro* protein digestibility, anti-nutrient and sensory properties of the products were evaluated. Chemical compositions of the biscuit samples revealed that sample E was significantly higher ($p < 0.05$) in ether extract (30.16%) and ash (4.20%) while sample F had a significantly higher protein content of 11.41%. The weight of the biscuit samples increased with increasing substitution levels with *Moringa* seed flour from 9.73–12.08 g. The heights of the samples were not affected by substitution levels. *In-vitro* protein digestibility values of the biscuits showed remarkable improvement from 11.03% for the control sample to 69.03% for sample E. Anti-nutrient content of the formulated biscuits showed that oxalate had values ranging from 18.68–35.71mg/100g, phytate 0.61–9.21mg/100g, saponin 0.46–8.41%, trypsin inhibitor 2.31–6.80mg/100g, tannin 18.68–35.71

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mg/100g and cyanide 0.02–0.44mg/100g. Sensory evaluation scores showed decreasing values in flavour and overall acceptability with increased levels of substitution with *Moringa* seed flour (7.5–10%) in the biscuit formulation. Although, these substitution levels led to an improvement in protein content, fibre and protein digestibility of the biscuit samples.

Keywords: *Biscuits; Moringa seed flour; African walnut flour; in-vitro protein digestibility; anti-nutrients analysis.*

1. INTRODUCTION

African walnut is of the family *Eurphorbiaceae* and they are found mainly in Nigeria, Southern Camerouns and the Congo region. It is botanically known as *Tetracarpidium conophorum*, commonly known in Eastern Nigeria as “ukpa” and “asala” in Western and Northern Nigeria. It is a dry nut that is enclosed in a hard shell and the plant is cultivated primarily for its nuts which are cooked and consumed. The nuts could be white or brown on cracking the shell [1]. Nwaoguikpe et al. [2] reported the presence of phytochemicals in the nuts and a high polyphenol composition consisting of ellagic and gallic acid alongside other phenolic acids.

Moringa oleifera has been a widely grown specie of the genera *Moringaceae*, found in most parts of humid tropical Africa, Asia and the South America. The average height of the tree is from 5 – 10m and various names in different countries such as drumstick tree or horse radish tree [3]. The seeds bitter taste is attributed to alkaloids, saponins and cyanogenic glycosides. The relative lack of anti-nutritional factors, availability of polyunsaturated fatty acids, high protein and sulphur containing amino acids encourage the use of *Moringa oleifera* as an animal feed. *Moringa oleifera* seeds contain essential amino acids in higher quantities than the WHO recommended dietary allowance [4].

Anti-nutrients such as tannins, saponines, oxalates, phytates, trypsin inhibitors and cyanides are natural or synthetic compounds that interfere with the absorption of nutrients. Tannins have been reported to be secondary metabolites of plants which are classified based on their structure into four groups viz: Gallotannins, Ellagitannins, Complex tannins and Condensed tannins [5]. Oxalates are a group of anti-nutrient compounds which contain oxalic acid and they combine with divalent metal cation such as calcium and iron to form crystals of corresponding oxalates [6].

Phytates are the major storage of phosphorus, containing 1–5% by weight in cereals, legumes, oil seeds and nuts, representing about 50–80% of total phosphorus in plants. In leguminous seeds and oil seeds, it is found within protein bodies while in cereal grains, it occurs in bran fraction and endosperm in the corn [7].

Saponins are nitrogen free glycosides, each containing a sapogenin and a sugar. The sapogenin may be a steroid or a triterpene and the sugar moiety is generally glucose, galactose, pentose or methy pentose. Tarade et al. [8] reported that saponins are responsible for the characteristic bitter or astringent taste found in most seeds.

Consumption of biscuits and similar foods made from wheat has become so popular in Nigeria that its total elimination from the dietary pattern could have nutritional and socio economic implications. The concepts of composite flours in food product formulation are not new and have been subject of numerous studies. Consumption of bakery products such as cakes and biscuit supplemented with African breadfruit flour, African walnut flour, locust bean flour and other underutilized plant seeds have been found to be beneficial in terms of improving the functional value of biscuits.

There have been several attempts at partial substitution of wheat flour with flour from readily available, cheap, indigenous tree crops like African walnut (*Tetracarpidium conorporum*). Nutritional studies have indicated that the seed of the African walnut is a potential source of macro nutrients and trace elements [9-11]. Biscuits currently sold in Nigeria have limited amounts of nutrients. The need for a product that has the potential to supply most of the nutrients needed and also meet the health challenges of a number of people is required. The study aimed at producing biscuit from composite flour blends of wheat, African walnut and *Moringa* seed and evaluate the chemical, physical, *in-vitro* protein digestibility, anti-nutrients and sensory characteristics of the biscuit samples.

2. MATERIALS AND METHODS

2.1 Materials

Mature African walnut (*Tetracarpidium conorophorum*) (raw seeds) were purchased from Abala Autonomous community in Obi-Ngwa LGA, Abia State while *Moringa* seeds (*Moringa oleifera*) were procured from Gaiya farms Ltd, Zaria, Kaduna State. Wheat flour (All purpose flour), granulated sugar (Dangote), margarine (Simas), salt (Dangote), baking powder (Longman), whole egg and vanilla flavour (Foster Clarks) were purchase from Next-Time Supermarket in Port Harcourt. All were transferred for processing and analysis to the Department of Food Science and Technology Laboratory, Rivers State University, Port Harcourt, all in Nigeria. All chemicals used were of analytical grade and were obtained from the same Department.

2.2 Methods

2.2.1 Preparation of African Walnut Flour (AWF)

African walnut kernel (*Tetracarpidium conorophorum*) flour was prepared using the method described by Barber and Obinna-Echem [12], with some modifications as shown in Fig. 1. African walnut seeds were sorted according to uniform size by the removal of small sized nuts and to remove extraneous material. The nuts were then washed and boiled in water ratio 3:1 (w/v) for 90 min, drained and allowed to cool at room temperature. Cracking and de-hulling were then carried out manually.

Particle size reduction was carried out by slicing the seeds into smaller dimensions of 1cm in diameter and dried with an electric oven at a temperature of 54°C for 6hr to allow for complete drying and ease of milling. The dried kernel were milled to finer particles that will pass through a 250µm sieve aperture size and packaged.

2.2.2 Preparation of *Moringa* seed flour

Moringa seed flour was prepared as described by Ogunsina et al. [13] with some modifications as shown in Fig. 2. Dried *Moringa* seeds were separated according to size, colour and shape and to remove extraneous materials and de-hulled manually and the husks discarded. The seeds were boiled in water 3:1 (v/w), drained and

dried at a temperature of 54°C for 4hr, allowed to cool to room temperature and then milled to flour.

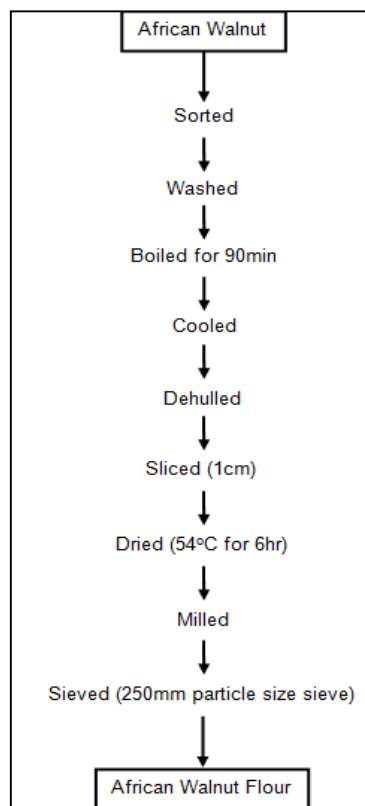


Fig. 1. Preparation of African walnut flour

Source: Barber and Obinna-Echem [12]

2.2.3 Biscuit production

Biscuit was produced adhering to the procedure of Agu and Okoli [14], with some modifications as shown in Fig. 3. Flour blends, sugar, margarine and baking powder were manually mixed in a mixing bowl to achieve uniformity, one whipped whole egg and 5ml of vanilla flavour were added and mixed thoroughly again for about 10min and 10ml of water was gradually added during mixing of the dough to aid the activation of the wheat gluten. The batter was rolled on a baking table with the aid of a roller. Cutting of the biscuits was done with a biscuit cutter and transferred to aluminium baking trays, whose surfaces have been previously lubricated with margarine to prevent burning. The surfaces of the biscuits were glazed with whipped egg. This was then transferred to a pre-set oven at 180°C and baked for 25min. The baked biscuits were then removed from the oven, allowed to cool before packaging.

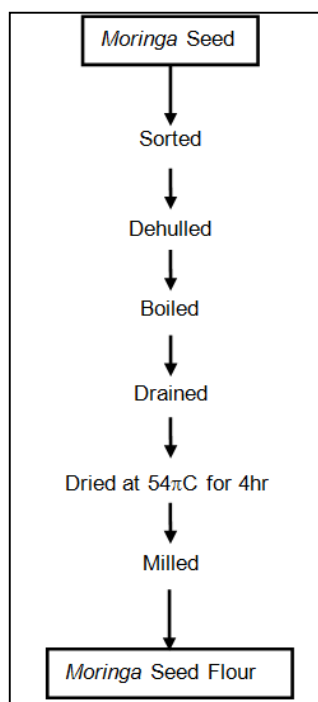


Fig. 2. Preparation of Moringa seed flour
Source: Ogunsina et al. [13]

2.2.4 Physicochemical analysis

The chemical compositions of the biscuit samples such as moisture, crude protein, fat, crude fibre and total ash were determined using AOAC [15] standard method and total carbohydrate was determined using Clegg Anthrone method as described by Osborne and Voogt [16]. Physical characteristics of the biscuits such as weight, diameter, height and spread ratio were calculated using the method described by Gomez et al. [17].

2.2.5 In-vitro protein digestibility

The *in-vitro* protein digestibility of the biscuit samples were determined using the method described by Saunders et al. [18] and modified by Monsour and Yusuf [19]. A known weight of each sample containing 16 mg nitrogen was taken and digested with 1mg of pepsin (Cat. No. P6887, Sigma Chemicals Ltd, USA) in 15ml of 0.1M HCl at 37°C for 3hr, the pepsin hydrolysed solutions were neutralized with 0.5M NaOH and incubated with 6.0 mg of pancreatin (Sigma Chemicals Ltd, Cat. No. P 1750) in 7.5 ml of 0.2 M phosphate buffer at pH 7.6 for 18hr. The reaction was terminated by adding 22.5ml of 10% TCA. The TCA soluble fraction was

assayed for nitrogen using the micro Kjeldahl method (Foss Teactor, model 2001).

A blank sample was also determined; protein digestibility was calculated using the formula:

$$\text{Protein digestibility} = \frac{\text{Nitrogen in supernatant} - \text{Nitrogen in blank}}{\text{Nitrogen in sample}} \times 100 \%$$

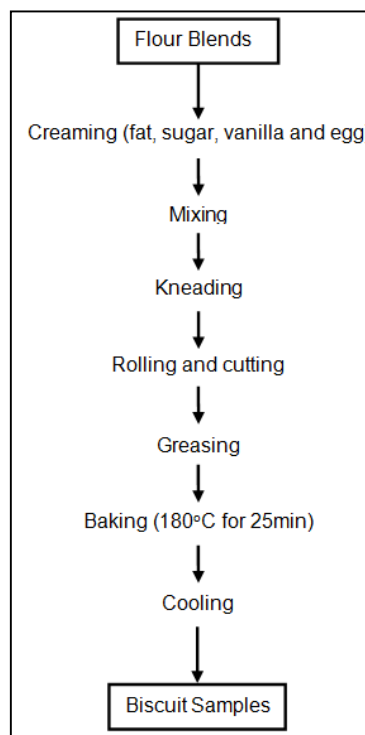


Fig. 3. Flow chart for the production of biscuit
Source: Agu and Okoli [14]

2.2.6 Determination of anti-nutrients

2.2.6.1 Tannins

Tannin content determination was carried out using the Folin-Ceocateu method described by Jaff [20]. Tannic acid standards 20, 40, 60, 80, 100 and 120 mg/l were prepared. Absorbance of various solution was read at 725 nm using a spectrophotometer. A calibration curve of the tannic acid was drawn and the concentration of the sample taken.

2.2.6.2 Oxalate

Oxalate content was determined using the method of Munro [21]. One gram (1g) of the sample was weighed into a 250ml conical flask and 75ml of 3N H₂SO₄ added. This was filtered

Table 1. Recipe formulation for the production of biscuit from WHF, AWF and MSF blends

Ingredients	Composite biscuit samples						
	A	B	C	D	E	F	G
Wheat flour (g)	100	77.7	75	92.5	70	90	80
Walnut flour (g)	0	20	20	20	20	0	20
Moringa flour (g)	0	2.5	5	7.5	10	10	0
Sugar (g)	30	30	30	30	30	30	30
Margarine (g)	45	45	45	45	45	45	45
Salt (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Baking powder (g)	2	2	2	2	2	2	2
Egg (whole)	1	1	1	1	1	1	1
Vanilla (ml)	5	5	5	5	5	5	5
Water	10	10	10	10	10	10	10

KEY: WHF = Wheat Flour, AWF = African walnut flour and MSF = Moringa seed flour; Samples A (WHF:AWF:MSF) = 100:0:0, B = 77.5:20:2.5, C = 75:20:5, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10 and G = 80:20:0

and 25 ml of filtrate was boiled, after 2 drops of methyl red indicator added and titrated hot against 0.05 M KMnO_4 solution until a pale pink colour persist for at least 30 seconds. The oxalate content was calculated by taking 1ml of 0.05 M KMnO_4 as equivalent to 2.2 mg oxalate.

$$\text{Oxalate (mg/100 g)} = \frac{\text{Titre value} \times 2.2\text{mg} \times \text{DF}}{\text{Wt of sample}}$$

2.2.6.3 Phytate

This was determined using dilute HCl and FeCl_3 as described by Essien and Akpan [22].

2.2.6.4 Saponins

Total Saponins was carried out according to the method described by Obadonin and Ochuko [23].

2.2.6.5 Cyanide

Cyanide content of the samples were determined using the alkaline picrate method as described by Onwuka [24].

2.2.6.6 Trypsin inhibitor

The Prokopet and Unlenbruck [25] method was used in the determination of Trypsin inhibitor. Standard concentrations (1mg, 2mg, 4mg, 6mg, 8mg and 10mg/l) trypsin were prepared and absorbance measured at 410 nm using a spectrophotometer.

2.2.7 Sensory evaluation

Sensory evaluation of the formulated biscuits were carried out using the method described by Giami and Barber [26]. The biscuit samples were presented to 20 panellists which comprises the students and staff of the Food Science and

Technology Department, Rivers State University on a white disposable flat plates and bottled water were also served for cleaning the mouth in-between each sample to prevent the transfer of sensory attributes from one sample to the other. Sensory attributes evaluated included colour, flavour, texture and overall Acceptability using 9 point hedonic scale ranging from 1 to 9; where 1 represented dislike extremely and 9 like extremely.

2.3 Statistical Analysis

Data obtained were analysed using Analysis of Variance (ANOVA) and means separated with the aid of Duncan Multiple range test at 5% level of probability using Statistical Package for Social Science (SPSS), version 20.0, year 2011.

3. RESULTS AND DISCUSSION

3.1 Chemical Compositions of Biscuit Samples Produced from Wheat Flour (WF), African Walnut Flour (AWF) and Moringa Seed Flour (MSF) Blends

Result of the study revealed that moisture content of the biscuit samples ranged from 6.56 to 7.80% with sample A being significantly higher ($p \geq 0.05$) with a value of 7.80% while total ash ranged from 1.35% for sample C to 1.76% for sample F as presented in Table 2. Values for ether extract indicated that control sample was the least (17.85%) and sample E (30.16%) the highest significantly. The protein content of the formulations ranged from 7.91 to 11.41% for samples A (0% MSF inclusion) and F (10% MSF inclusion), respectively. This result is in agreement with that of Emelike et al. [27] who reported that the protein value of cookies

produced from 0% egg and 5% *Moringa* leaf flour compared favourably with those produced from 5% egg and 0% *Moringa* leaf flour. Kiin-Kabari et al. [28] equally reported that substitution of egg with 10% *Moringa* leaf flour increased the protein value of the produced cookies than those from 0% *Moringa* leaf flour. This is in confirmation that inclusion of *Moringa* either the leaf or the seed up to 10% in the formulation of bakery foods have the capacity of improving its protein content. Protein is essential for growth, building body blocks and for repair of damaged tissues [29]. Crude fibre content ranged from 0.99 (sample A) to 4.20% (sample E) while total carbohydrate values were from 49.29 to 61.92% for samples C and A, respectively. Okoye and Obi [30] reported that the result obtained in their study revealed that various levels of wheat and African breadfruit composite flours in the production of cookies affected the nutrient compositions of the products.

3.2 Physical Characteristics of Biscuit Samples Produced from Wheat Flour (WHF), African Walnut Flour (AWF) and *Moringa* Seed Flour (MSF) Blends

Physical characteristics result revealed that sample A had the least mean weight of 8.93 g followed by sample F 9.02 g, G 9.44 g, B 9.73 g, C 10.18 g, D 11.06 g and E 12.08 g as the highest with significant difference ($p \leq 0.05$) in all the samples as shown in Table 3. The mean diameter of the biscuit samples had range values of 4.45 cm to 5.02 cm for samples E as the least and A the highest, significantly. Samples B, C, E and F were not significantly different ($p \leq 0.05$) while significant difference existed within other samples.

The result revealed that increasing addition of *Moringa* seed flour and a decreasing wheat flour in cookie formulation led to a continuous decrease in the height of the cookie samples with samples A having the highest height value (0.79 cm) and E with the lowest value of 0.61 cm. Reduction in height and diameter of composite cookies have been similarly reported by Giami and Barber [26] for cookies made from fluted pumpkin flour. This similar report could be attributed to the gluten content of wheat flour which is responsible for the proofing characteristics of dough. The height of samples B and G with 2.5% and 0% *Moringa* seed flour, respectively compared favourably with sample A (100% wheat flour). This correlated with spread ratio as samples A and G compared suitably with each other while unstable variations was observed in the spread ratio of other samples. Although, there was no significant difference ($p \leq 0.05$) in the spread ratio of samples B, D and E. Chinna et al. [31] reported slight increase in weight in composite flour of defatted sesame seed and unripe plantain flour while spread ratio decreased. This report is in agreement with the one observed in this present study.

3.3 In-vitro Protein Digestibility of Biscuit Samples Produced from Wheat Flour (WHF), African Walnut Flour (AWF) and *Moringa* Seed Flour (MSF) Blends

The result revealed that increasing substitution levels of *Moringa* seed flour led to an increasing protein digestibility values of the biscuit samples as presented in Table 4. Sample A with 100% WHT had the lowest protein digestibility value of 11.03% while sample E with 20% AWF and 10% MSF substitution levels had significantly highest

Table 2. Chemical compositions of the biscuit samples produced from WHF, AWF and MSF flour blends

Samples	Moisture (%)	Total ash (%)	Ether extract (%)	Crude protein (%)	Crude fibre (%)	Total carbohydrate (%)
A	7.80 ^a ±0.00	1.47 ^c ±0.00	17.85 ^f ±0.02	7.91 ^e ±0.11	0.99 ^e ±0.02	61.92 ^a ±0.05
B	6.56 ^c ±0.11	1.61 ^b ±0.12	28.12 ^b ±0.14	8.76 ^d ±0.34	1.29 ^d ±0.00	51.74 ^b ±0.67
C	7.26 ^b ±0.23	1.35 ^c ±0.18	27.88 ^c ±0.04	10.37 ^b ±0.25	1.55 ^d ±0.19	49.29 ^d ±1.26
D	7.49 ^b ±0.07	1.61 ^b ±0.14	26.46 ^d ±0.38	10.39 ^b ±0.08	3.19 ^b ±0.00	49.83 ^d ±0.15
E	7.41 ^b ±0.23	1.38 ^c ±0.09	30.16 ^a ±0.79	9.97 ^c ±0.08	4.20 ^a ±0.14	52.06 ^d ±0.20
F	7.04 ^c ±0.16	1.76 ^a ±0.02	20.44 ^e ±0.01	11.41 ^a ±0.14	2.42 ^c ±0.05	56.91 ^b ±0.13
G	7.33 ^b ±0.06	1.25 ^c ±0.18	25.24 ^d ±0.29	9.14 ^c ±0.42	2.13 ^c ±0.00	54.90 ^c ±0.01

Means with different superscripts within a column are significantly different ($p \geq 0.05$), ± Standard Error (SE) of triplicate determination; KEY: WHF = Wheat Flour, AWF = African walnut flour and MSF = *Moringa* seed flour Samples A (WHF:AWF:MSF) = 100:0:0, B = 77.5:20:2.5, C = 75:20:5.0, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10 and G = 80:20:0

Table 3. Physical characteristics of biscuit samples produced from WHF, AWF and MSF blends

Sample	Weight (g)	Diameter (cm)	Height (cm)	Spread ratio
A	8.93 ^d	5.84 ^a	0.79 ^a	7.39 ^a
B	9.73 ^e	4.90 ^c	0.75 ^a	6.52 ^d
C	10.18 ^c	4.83 ^c	0.72 ^b	6.67 ^c
D	11.06 ^b	4.61 ^d	0.71 ^b	6.49 ^d
E	12.08 ^a	4.45 ^c	0.61 ^c	6.53 ^d
F	9.02 ^g	4.48 ^c	0.63 ^c	7.11 ^b
G	9.44 ^f	5.02 ^b	0.77 ^a	7.30 ^a

Mean values with different superscripts within a column are significantly different ($p \geq 0.05$); KEY: WHF = Wheat Flour, AWF = African walnut flour and MSF = Moringa seed flour; Samples A (WHF:AWF:MSF) = 100:0:0, B = 77.5:20:2.5, C = 75:20:5.0, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10 and G = 80:20:0

protein digestibility value of 69.03%. High protein digestibility values recorded in biscuit samples produced from the substitution levels of AWF and MSF could be associated with the presence of soluble protein fraction such as albumin which is easily hydrolysed by enzymes. Another factor could be the unfolding of native protein structure which contributes to digestibility. The result of this study is in agreement with the report of Kiin-Kabari and Giami [32], they also recorded high *in-vitro* protein digestibility of 62.81% in cookie samples containing bambara groundnut protein concentrate.

3.4 Anti-nutrients

The anti-nutrient contents of biscuit samples produced from wheat flour (WHF), African walnut flour (AWF) and *Moringa* seed flour (MSF) blends are shown in Table 5. Results revealed an increase in the levels of tannin from sample A to E (18.68mg/100g – 35.71mg/100g). The oxalate values also increased with substitution levels of the flour samples. Sample A and E had significantly lowest and highest values of 61.92mg/100g and 114.09mg/100g, respectively and all the oxalate values were significantly different from each other. The phytate, saponin, trypsin inhibitor and hydrogen cyanide values of the prepared biscuit samples were lower than those reported by Ekwe and Ihemeje [33] for African walnut flour. Results obtained showed that the cyanide content of the biscuit samples was the least amongst the anti-nutrients. The concentration of the African walnut in the biscuit samples showed that there were no significant differences in the hydrogen cyanide values between samples B, C, D and E while there were significant difference between samples A, F and G. The percentage concentration of saponin in the biscuits though low, also increased with substitution levels. Saponin content of the biscuits ranged from 0.61-8.41%. The tannin

content of 450 mg/100 g was lower than those reported by Fasoyiran et al. [34] for groundnut seeds.

Table 4. *In-vitro* protein digestibility of biscuits produced from WHF, AWF and MSF blends

Sample	Protein digestibility (%)
A	11.03 ^f ±1.00
B	49.87 ^c ±0.85
C	56.15 ^b ±1.57
D	60.04 ^b ±3.27
E	69.03 ^a ±0.87
F	23.77 ^e ±1.03
G	39.46 ^d ±2.62

Means with different superscripts within a column are significantly different ($p \geq 0.05$), \pm SE of duplicate determination; KEY: WHF = Wheat Flour, AWF = African Walnut Flour, MSF = Moringa Seed Flour Samples A (WHF:AWF:MSF) = 100:0:0, B = 77.5:20:2.5, C = 75:20:5.0, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10 and G = 80:20:0

3.5 Sensory Evaluation

The sensory evaluation of the biscuit samples produced from wheat flour (WHF), African walnut flour and *Moringa* seed flour (MSF) blends is presented in Table 6. The quality parameters analysis indicated that sample A produced with 100% WHF had significantly highest ($p \leq 0.05$) values in terms of colour 8.20, flavour 8.10 and texture 7.85 while there was a significant relationship between samples A, B and G with respect to the overall acceptability of the biscuit samples. Results equally indicated that increasing substitution levels of *Moringa* seed flour decreased the overall acceptability scores of the biscuit samples. Hence, acceptable biscuit that compares well the control sample can successfully be produced with either ratios of 77.5:20:2.5 or 80:20:0 (WHF:AWF:MSF).

Table 5. Anti-nutrient content of biscuit samples produced from WHF, AWF and MSF blends

Parameters	A	B	C	D	E	F	G
Tannin (mg/100 g)	18.68 ^g ±0.04	25.76 ^d ±0.20	26.44 ^c ±0.25	27.21 ^b ±0.01	35.71 ^a ±0.44	20.61 ^f ±0.01	23.02 ^e ±0.01
Oxalate (mg/100 g)	61.92 ^g ±0.85	99.49 ^d ±2.82	101.13 ^c ±0.06	109.04 ^b ±0.76	114.09 ^a ±0.20	72.93 ^f ±0.08	98.04 ^e ±0.20
Phytate (mg/100 g)	0.61 ^g ±0.00	2.32 ^a ±0.11	1.60 ^c ±0.04	9.21 ^f ±0.06	1.36 ^d ±0.02	1.05 ^e ±0.36	1.89 ^b ±0.01
Saponin (%)	0.46 ^e ±0.01	1.01 ^c ±0.02	2.43 ^b ±0.14	2.26 ^b ±0.06	8.41 ^a ±0.03	0.32 ^f ±0.08	0.61 ^d ±0.01
T. Inhibitor (mg/100 g)	2.31 ^e ±0.01	4.60 ^b ±0.02	3.75 ^d ±0.04	3.78 ^d ±0.01	3.91 ^c ±0.02	1.31 ^f ±0.10	6.80 ^a ±0.00
HCN (mg/100 g)	0.02 ^d ±0.00	0.40 ^a ± 0.03	0.42 ^a ±0.03	0.40 ^a ±0.01	0.44 ^a ±0.03	0.14 ^c ±0.00	0.31 ^b ±0.01

Means with different superscripts within a row are significantly different ($p \leq 0.05$), \pm SE of triplicate determination; KEY: HCN = Hydrogen Cyanide; WHF = Wheat Flour, AWF = African Walnut Flour, MSF = Moringa Seed Flour; Samples A (WHF:AWF:MSF) = 100:0:0, B = 77.5:20:2.5, C = 75:20:5.0, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10 and G = 80:20:0

Table 6. Sensory evaluation of the biscuit samples produced from WHF, AWF and MSF blends

Sample	Colour	Flavour	Texture	Overall acceptability
A	8.20 ^a ±0.20	8.10 ^a ±0.24	7.85 ^a ±0.20	8.17 ^a ±0.22
B	7.65 ^c ±0.27	7.60 ^c ±0.24	7.65 ^c ±0.23	7.80 ^{ab} ±0.23
C	7.45 ^d ±0.19	7.55 ^c ±0.17	7.20 ^d ±0.25	7.50 ^b ±0.14
D	6.65 ^e ±0.23	6.60 ^d ±0.24	6.60 ^e ±0.24	6.65 ^d ±0.18
E	6.30 ^f ±0.24	6.25 ^f ±0.21	6.35 ^f ±0.26	6.25 ^e ±0.19
F	7.83 ^b ±0.14	6.50 ^e ±0.03	7.74 ^c ±0.07	7.02 ^c ±0.02
G	7.80 ^b ±0.20	7.95 ^b ±0.24	7.80 ^b ±0.20	8.10 ^a ±0.22

Means with different superscripts within a row are significantly different ($p \leq 0.05$), \pm SE of triplicate determination

KEY: WHF = Wheat Flour, AWF = African Walnut Flour, MSF = Moringa Seed Flour; Samples A (WHF:AWF:MSF) = 100:0:0, B = 77.5:20:2.5, C = 75:20:5.0, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10 and G = 80:20:0

4. CONCLUSION

Proximate compositions of the flour blends increased with increase in substitution levels with *Moringa* seed flour and African walnut flour. Samples B and G compared significantly with sample A in terms of height while sample G compared significantly with sample A with regards to the spread ratio of the biscuit samples. Increase in substitution levels of the flour blends led to a corresponding increase in protein digestibility of the products and highest increase was achieved at 70:20:10 (WHF:AWF:MSF) blends. Substitution levels also led to a continuous increase in tannin, oxalate and saponin contents of the biscuit samples while a fluctuation was observed in the values of other anti-nutrients. Product acceptability decreased along with substitution levels of African walnut and *Moringa* seed flours. Although, the overall acceptability of samples B and G compared favourably with the control product.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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