



Research article

Physicochemical properties, *in vitro* digestibility, antioxidant activity and consumer acceptability of biscuits prepared from germinated finger millet and Bambara groundnut flour blends

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ARTICLE INFO

Keywords:

Cereal
Biscuit
Gluten-free
Antioxidant
Antinutrients
Texture

ABSTRACT

The formulation of new food products with high nutritional quality and functionality is gaining global attention. The physicochemical properties, *in vitro* digestibility, antioxidant activity and consumer acceptability of biscuits produced from germinated finger millet (GFM) (*Eleusine coracana*) and Bambara groundnut (GBGN) (*Vigna subterranea*) flour blends were investigated. As the proportion of GBGN flour increased in the biscuit samples, protein, *in vitro* protein digestibility (80.52–89.20 %), slowly digestible and resistant starch, total phenolic content and antioxidant activities increased significantly, while rapidly digestible starch, starch hydrolysis index, glycemic index and phytic acid decreased. Addition of GBGN also positively influenced the physical attributes of the biscuits. The blending of 80% GFM with 20 % GBGN resulted in a biscuit with acceptable sensory qualities such as taste, aroma, appearance, crunchiness, and overall acceptability. This study showed that GFM and GBGN flour blends could serve as functional ingredients to produce better products.

1. Introduction

The increasing interest of consumers for functional foods and gluten-free products have encouraged the inclusion of novel plant food materials in food product development. Biscuits can serve as good vehicle to dispense essential nutrients and health promoting compounds, in addition to the fact that such snacks are affordable, convenient, and generally accepted by the populace (Di Cairano et al., 2018). Gluten-free biscuits

can be prepared from bioprocessed whole grain cereals or combination of cereals and legumes that may improve the nutritional and health-promoting characteristics as well as consumer acceptability of the biscuits (Adebiyi et al., 2017; Di Cairano et al., 2018).

Finger millet (*Eleusine coracana*) is an underutilized gluten-free grain with high nutritional quality, rich sources of phenolics and bioactive compounds and could serve as a healthy food ingredient (Ramashia et al., 2017; Xiang et al., 2019) and commonly used in the preparation of

This article is a part of the "Unconventional sources of food and food ingredients" Special issue.

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<https://doi.org/10.1016/j.heliyon.2022.e10849>

Received 15 April 2022; Received in revised form 3 July 2022; Accepted 27 September 2022

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porridges, non-alcoholic beverages, and baked products (Adebiyi et al., 2017). Bambara groundnut (*Vigna subterranea*) is also an underutilized plant protein source grown in many parts of sub-Saharan Africa and Southeast Asia (Oyeyinka et al., 2018). The chemical composition (on dry basis) of Bambara groundnut consists of 17–27% protein (with adequate balance of amino acids), 3.3–4.4% crude fiber, 5.5–6% fat, 60–63% carbohydrate and high contents of micronutrients (Oyeyinka et al., 2018). As such, Bambara groundnut flour can serve as an important ingredient to improve the nutritional profile of gluten-free bakery products (Di Cairano et al., 2018).

Germination is a traditional inexpensive bioprocessing food processing technique that can reduce antinutritional factors, modify nutrient and health promoting constituents as well as sensory properties and consumer acceptability (Adebiyi et al., 2017; Chinma et al., 2021). Considering the potential of this processing technique, it could positively modify the composition of these underutilized grains and improve their possibility for subsequent food preparation. The use of germinated finger millet (GFM) and Bambara groundnut (GBGN) flours in the preparation of biscuits may thus increase the nutrient density of gluten-free biscuits in the market and reduce the high burden of micronutrient deficiency in the developing regions of the world. The study was thus aimed at evaluating the physicochemical properties, *in vitro* digestibility, antioxidant activity and consumer acceptability of biscuits prepared from germinated finger millet and Bambara groundnut flour blends.

2. Materials and methods

2.1. Materials

Both the brown variety Finger millet grains and Bambara groundnut (BGN) seeds were procured from a seed Company based in Kaduna, Nigeria. The BGN and brown FM seeds were sorted, cleaned and stored in airtight containers at ambient condition till subsequent use.

2.2. Germination of finger millet

Finger millet (FM) grains and BGN seeds were cleaned, treated with 0.07 g/L food grade NaClO for 30 min. This was then drained, followed by soaking in distilled water (1:5 w/v) at room temperature for 6 h. The moistened FM grains and BGN seeds were then germinated for 72 h at 25 °C. Uniformly germinated seeds and grains were selected and separately dried at 40 °C in an oven (Gallenkamp 300 plus series, Widnes, Cheshire, UK) for 24 h. The dried FM grains and BGN seeds were separately milled into flour and passed through 100µm sieve to obtain GFM and GBGN flour.

2.3. Biscuit preparation

The 100% GFM and GFM-GBGN composite flour samples were used in the preparation of biscuit following the modified method of Adebiyi et al. (2017). Different proportions (100:0, 90:10, 80:20, 70:30 and 60:40) of GFM and GBGN flours were blended (BLX750RD, Kenwood, Sheffield, UK) in order to achieve uniform mixing. The biscuit formulation comprised of 225 g flour, 56 g sugar (Dangote Refinery Plc, Lagos, Nigeria), 13.5 g of vanilla essence (Vanilla, Gim Hin Lee, India), 66 g of shortening (Blue Band, Unilever Plc., Lagos, Nigeria), 1.5 g of baking powder (Bake's choice, Graceco Limited, Alagbado, Nigeria) and 120 mL of water. The dough was rolled into a thickness of 5.80 mm and cut into round shapes using a biscuit cutter. The biscuits were baked in pre-heated oven (Gallenkamp, UK) at 180 °C for 20 min. Triplicate biscuit samples were prepared and were subsequently analyzed.

2.4. Proximate analysis of flour and biscuits

Moisture, protein, ash and fat were assayed by the AOAC methods of 925.09, 992.23, 923.03 and 920.39 (AOAC, 2005), respectively. The

total dietary fiber and total starch contents were analyzed by the AOAC international methods of 32–45.01 and 76–13.01 (AOAC International, 2015), respectively. Carbohydrate content (calculated by difference) and energy value (calculated based on 4.0 kcal/g for protein and carbohydrate, and 9.0 kcal/g for fat) was determined following a standard method (AOAC, 2005).

2.5. Antinutritional factors in flour and biscuits

The concentration of phytic acid (PA) was profiled based on AOAC (2005) method, using a UV-spectrophotometer (Genesys G10S, Thermo Fisher Scientific, Waltham, USA) at 640 nm and values expressed on dry weight basis as mg/100 g. Trypsin inhibitor activity was assayed using 0.04 % (w/v) of BAPA (N α -benzoyl-L-arginine 4- nitroanilide hydrochloride) as trypsin substrate (Liu and Markakis, 1989). Measurements were made using a UV-spectrophotometer at 410 nm and values expressed as trypsin inhibitor (TI) unit per mg (dw).

2.6. Mineral analysis of flour and biscuits

Previously ashed samples (section 2.4) were used in the determination of mineral content of the samples using atomic absorption spectroscopy (AAS) (PerkinElmer Model 2380, USA) following the standard conditions detailed for each mineral element by the manufacturer of the AAS.

2.7. Total phenol and antioxidant activities of flour and biscuits

Extracts were obtained using 80% methanol (Chinma et al., 2014) and the methanolic extract (ME) were subsequently used for the analyses. The total phenolic content was measured with a UV-spectrophotometer, following the method of Singleton & Rossi (1965) and the values presented on dry basis as mg gallic acid equivalents (GAE)/100 g. The ferric reducing antioxidant power (FRAP) of the samples was assayed using the method of Beta et al. (2005) and results obtained defined on a dry basis as µmol trolox equivalents (TE)/100 g. Free radical scavenging ABTS assay was determined using the method of Re et al. (1999) and results were expressed on dry basis as mg TE/100 g.

2.8. *In vitro* protein digestibility analysis of flour and biscuits

The *in vitro* protein digestibility (IVPD) was determined by weighing 200 mg of sample into Erlenmeyer flask (100 mL) that contained 35 mL sodium citrate tribasic dihydrate (pH 2.0 and 0.1 mol/L) with 1.5 g pepsin/L (Ojokoh and Yimin, 2011). Thereafter, the mixture was incubated for 2 h in a water bath (NLS420S, Genlab Ltd., Cheshire, UK) at 37 °C. This was followed by centrifugation (K24IR, Centurion Scientific Ltd, Chichester, UK) at 10,000 × g for 15 min. The supernatant was then removed and subsequent residue obtained washed, dried and analyzed for nitrogen content following the procedure of AOAC (2005). The IVPD was then computed as percentage of protein in supernatant / total protein content of the sample.

2.9. *In vitro* digestibility of starch in flour and biscuits

Parameters of starch hydrolysis of each biscuit sample was determined using the procedures elucidated by Goñi et al. (1997). Accordingly, Eq. (1) was used to calculate the percentage of hydrolyzed starch;

$$C = C_{\infty} - (1 - e^{-kt}) \quad (1)$$

where C is the percentage of hydrolyzed starch at time t , C_{∞} is the equilibrium hydrolyzed starch after 180 min and k is the kinetic constant).

Thereafter, the hydrolysis index (HI) of the products were obtained (by dividing the areas under the hydrolysis curve of each sample). From

the HI value, the estimated glycemic index (eGI) of the samples was obtained using Eq. (2) (Goñi et al., 1997):

$$eGI = 39.7 + 0.548HI \quad (2)$$

where eGI = estimated glycemic index (%); HI = hydrolysis index (%).

The rapidly digested starch (RDS, hydrolyzed at 20 min) slowly digested starch (SDS, hydrolyzed between 20 and 120 min) and resistant starch (RS, undigested after 120 min) were also determined.

2.10. Physical properties of biscuits

The thickness and diameter of biscuits prepared from each blend were measured with a vernier caliper in two perpendicular directions and average results were reported (Korus et al., 2017). The spread ratio of the biscuit was determined by dividing biscuit diameter by the thickness (Korus et al., 2017). Colour attributes of the biscuits were measured using a Chroma-Meter (CR-410, Konica-Minolta, Japan) and the L* (lightness), a* (redness) and b* (yellowness) values were recorded. The texture (hardness) of the biscuit samples were measured at 28 ± 1 °C using an Instron universal testing machine (model 3342; Instron, USA), with a load cell of 50 N (Bourne, 1978).

2.11. Small-scale consumer test

Preceding this test, ethical approval was obtained from Research Ethics Committee, Federal University of Technology Minna, Nigeria. Consent of the sensory panelists was also sort and received. Subsequent small-scale sensory assessment of the biscuit was conducted immediately after baking using the method of Roncolini et al. (2020) using 20 semi-trained panelists (comprising of students and staff, who are regular biscuit consumers that aged between 20 to 35 years). The panelists assessed the samples under white fluorescent light in individual booths. The coded samples were presented randomly as follows: MXG (70GFM:30GBGN), XAL (90GFM:10GBGN), MEX (60GFM:40GBGN), PXN (100GFM) and MON (80GFM:20GBGN), in white plastic plates. The panelists then evaluated them using a 9-point Hedonic scale (where 9 represent like extremely and 1 denote dislike extremely) for aroma, taste, appearance, crunchiness, and overall acceptability. The panelists were provided with drinking water to rinse the mouth between evaluations.

2.12. Statistical analysis

Each analysis was conducted in triplicates. Results obtained were expressed as the mean \pm standard deviation. The least significant difference (LSD) test was used to determine significant differences at 5% probability level using a statistical software (SPSS version 16, IBM, Armonk, USA).

3. Results and discussion

3.1. Proximate and antinutritional composition of biscuits

The proximate composition and antinutritional composition (dry basis) of the flour and biscuits produced from GFM and GBGN blends are presented in Tables 1 and 2. Significantly higher values of protein (25.87 g/100g), ash (7.91 g/100g) and fat (5.52 g/100g), were recorded in the GBGN flours, compared to the GFM flours (Table 1). The moisture, ash, protein, fat, total dietary fiber, total carbohydrate, starch and energy value of biscuits produced from GFM, GBGN and their blends were 8.12–8.63 g/100 g, 1.34–2.32 g/100 g, 11.06–20.74 g/100 g, 6.24–8.90 g/100 g, 13.32–18.39 g/100 g, 35.28–42.50 g/100 g, 59.59–73.24 g/100 g and 393.36–401.42 kcal/100 g, respectively (Table 2). The moisture value of the biscuit increased following the increase in the level of GBGN (≥ 20 %) in the blend. This increase could be ascribed to the high-water absorption capacity of GBGN's proteins. Mashau et al. (2020) had attributed the increase in the moisture content of maize-based tortilla to the high-water absorption

Table 1. Chemical composition and antioxidant properties of germinated finger millet (GFM) and germinated Bambara groundnut (GBGN) flour.

Parameter	GFM	GBGN
Moisture (g/100 g)	10.37 \pm 0.22 ^a	9.82 \pm 0.34 ^b
Starch (g/100 g)	53.48 \pm 0.23 ^a	42.60 \pm 0.31 ^b
Ash (g/100 g)	4.96 \pm 0.05 ^b	7.91 \pm 0.40 ^a
Protein (g/100 g)	10.97 \pm 0.03 ^b	25.87 \pm 0.13 ^a
Fat (g/100 g)	3.37 \pm 0.27 ^b	5.52 \pm 0.02 ^a
Total dietary fiber (g/100 g)	20.92 \pm 0.56 ^a	17.81 \pm 0.75 ^b
Total carbohydrate (g/100 g)	50.59 \pm 0.36 ^a	33.07 \pm 0.24 ^b
Energy value (kcal/100 g)	276.57 \pm 1.62 ^b	285.44 \pm 1.30 ^a
In vitro protein digestibility (%)	78.33 \pm 0.81 ^b	87.60 \pm 0.90 ^a
Phytic acid (mg/100 g)	324.70 \pm 5.22 ^a	3.12 \pm 0.10 ^b
TIA (TIU/mg)	ND	1.58 \pm 0.03 ^a
Calcium (mg/100 g)	161.92 \pm 1.18 ^a	57.24 \pm 0.58 ^b
Iron (mg/100 g)	248.15 \pm 1.30 ^a	160.10 \pm 1.13 ^b
Magnesium (mg/100 g)	1356.20 \pm 6.19 ^a	132.55 \pm 1.39 ^b
Phosphorus (mg/100 g)	2694.57 \pm 4.04 ^a	309.40 \pm 1.82 ^b
Zinc (mg/100 g)	5.52 \pm 0.19 ^a	3.83 \pm 0.01 ^b
TPC (mg GAE/100 g)	164.03 \pm 1.16 ^b	205.20 \pm 1.09 ^a
FRAP (μ mol TE/100 g)	413.95 \pm 1.53 ^b	435.60 \pm 3.82 ^a
ABTS (μ mol TE/100 g)	251.83 \pm 1.16 ^b	301.48 \pm 1.73 ^a

Mean and standard deviation of triplicates. Mean value with different superscript in a row are significantly ($p \leq 0.05$) different from each other. TPC = Total phenolic content; FRAP = Ferric reducing antioxidant power; ABTS = 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) free radical scavenging assay; ND = Not detected, TIA = Trypsin inhibitor activity.

capacity of Bambara groundnut's proteins. The moisture content obtained for all the biscuit samples was generally low and this could imply good shelf stability as lower moisture content would limit the proliferation of micro-organisms. Besides, this could also contribute to improved textural and sensory qualities of the product (as noted in Sections 3.5 and 3.6). The ash, fat, protein and energy value of the product increased with an increasing proportion of GBGN while total dietary fiber, carbohydrate and starch content decreased. This could be ascribed to the high value of ash, protein and fat in GBGN while decreased dietary fiber, carbohydrate and starch content may be attributed to substitution effect (Table 1). This finding agreed with previous studies that have investigated the supplementation of BGN and GBGN in the development of food products with reported increases in protein, fat and ash contents (Adegunwa et al., 2017; Yeboah-Awudzi et al., 2018; Abdulrahman et al., 2019; Agu et al., 2020).

The protein, ash, dietary fiber and energy value results obtained in this study also showed that the biscuit produced from GFM had higher ash, protein, dietary fiber and energy value than the biscuit prepared from germinated pearl millet (Adebiyi et al., 2017). This suggests the high nutrient profile of germinated finger millet, and therefore, could be an appropriate raw material to produce value-added food products. For example, the control (GFM) and GFM-BGN composite biscuits could also be regarded as high fiber biscuits, since the biscuits contain between 13 - 18 g/100 g dietary fiber. The consumption of food rich in dietary fiber has numerous health benefits such as reduction in the risk of chronic diseases (including diabetes, cardiovascular diseases, obesity and types of cancer) in humans as well as promotion of gut microbiota growth which positively control several physiological activities in the body (Zhu, 2020). In addition, the reduction in starch content (from 42.50 to 35.28 g/100g, Table 2) of the biscuits with increasing GBGN level could suggest better nutritional advantage considering the physiological effects of high starchy foods in humans. The starch content of the composite biscuits is comparable to gluten-free biscuits prepared from buckwheat-millet-chickpea/lentil (50:30:20) which contained 36.04–38.63 g/100 g (Di Cairano et al., 2021) but relatively low compared to cookies prepared with different proportions of alfalfa seed flour (Giuberti et al., 2018). The energy value of the composite biscuits (396.32–401.42 kcal/100 g) were

Table 2. Proximate composition, phytic and trypsin inhibitor activity of biscuits.

Parameter	100GFM	90GFM: 10GBGN	80GFM: 20GBGN	70GFM: 30GBGN	60GFM: 40GBGN
Moisture (g/100 g)	8.12 ± 0.06 ^b	8.05 ± 0.10 ^b	8.63 ± 0.03 ^a	8.32 ± 0.11 ^{ab}	8.45 ± 0.08 ^a
Total starch (g/100 g)	42.50 ± 0.34 ^a	41.20 ± 0.13 ^b	40.64 ± 0.17 ^c	38.81 ± 0.65 ^d	35.28 ± 0.83 ^e
Ash (g/100 g)	1.34 ± 0.01 ^c	1.66 ± 0.02 ^d	1.94 ± 0.01 ^c	2.13 ± 0.05 ^b	2.32 ± 0.01 ^a
Protein (g/100 g)	11.06 ± 0.74 ^c	14.53 ± 0.15 ^d	17.87 ± 0.19 ^c	19.40 ± 0.50 ^b	20.74 ± 0.47 ^a
Fat (g/100 g)	6.24 ± 0.03 ^c	7.04 ± 0.27 ^d	7.73 ± 0.45 ^c	8.15 ± 0.33 ^b	8.90 ± 0.28 ^a
Total dietary fiber (g/100 g)	18.39 ± 0.26 ^a	17.50 ± 0.14 ^b	15.62 ± 0.21 ^c	14.27 ± 0.17 ^d	13.32 ± 0.23 ^e
Total carbohydrate (g/100 g)	73.24 ± 0.57 ^a	68.71 ± 0.39 ^b	63.83 ± 0.41 ^c	62.00 ± 0.22 ^d	59.59 ± 0.25 ^e
Energy value (kcal/100 g)	393.36 ± 1.10 ^d	396.32 ± 1.25 ^c	396.37 ± 1.02 ^c	398.95 ± 1.17 ^b	401.42 ± 1.30 ^a
Phytic acid (mg/100 g)	241.20 ± 1.03 ^a	203.75 ± 1.19 ^b	176.44 ± 1.26 ^c	125.52 ± 1.33 ^d	94.30 ± 0.77 ^e
TIA (TIU/mg)	ND	0.10 ± 0 ^a	0.18 ± 0 ^a	0.21 ± 0.01 ^a	0.24 ± 0.01 ^a

Mean and standard deviation of triplicates. Mean value with different superscript in a row are significantly ($p \leq 0.05$) different from each other. 100GFM = 100 % germinated finger millet; 90GFM:10GBGN = 90 % germinated finger millet and 10 % germinated Bambara groundnut flour; 80GFM:20GBGN = 80 % germinated finger millet and 20 % germinated Bambara groundnut flour; 70GFM: 30GBGN = 70 % germinated finger millet and 30 % germinated Bambara groundnut flour, 60GFM:40GBGN = 60 % germinated finger millet and 40 % germinated Bambara groundnut flour ND = Not detected, TIA = Trypsin inhibitor activity.

also comparable to the values (312.20–396.50 kcal/100 g) reported for acorn-hemp biscuits (Korus et al., 2017).

The phytic acid (PA) content (94.30–241.20 mg/100 g) of the biscuit reduced as the level of GBGN increased in GFM and GBGN flour blends. Trypsin-inhibitor activity (TIA) was not detected in the biscuit produced from 100 % GFM while generally low content of TIA (≤ 0.24 TIU/mg) was recorded in biscuits produced from the blends of GFM and GBGN. The low concentration of PA in biscuit samples that contained a high level of GBGN could indicate improved bioavailability of macro-minerals, especially Calcium. The PA and TIA of the samples were generally low compared to the flours (Table 1). The low concentration of the anti-nutrients in the biscuit samples could be due to their thermal degradation during baking. This would contribute to an increase in the digestibility and bioavailability of essential nutrients when consumed. The level of PA in the developed product is safe for human consumption, considering the reference daily intake (RDI) value of phytate of 631–746 mg RDI/day (for the USA and UK) (Nissar et al., 2017). Although PA can reduce mineral bioavailability; PA provide various health benefits such as antioxidant and anti-carcinogenic properties, amongst others (Campos-Vega et al., 2010).

The mineral profile of the biscuit samples showed that Ca, Fe, Mg, P, and Zn reduced with an increasing level of GBGN (Table 3). This could be due to the lower mineral profile of GBGN relative to GFM (Table 1). The mineral profile of biscuit from GFM was higher than the values reported

for the biscuit produced from germinated pearl millet (Adebiyi et al., 2017). Despite the reducing level of minerals with increasing GBGN level in the biscuit samples, the Fe (252.60 mg/100 g) and Mg (1372.34 mg/100 g) content of the sample produced from 60 % GFM and 40 % GBGN flour blend was higher than 138.62 and 286.15 mg/100 g, respectively reported for the biscuit produced from germinated pearl millet (Adebiyi et al., 2017) as well as multigrain gluten-free biscuits (45.28 mg/100 g calcium, 3.47 mg/100 g iron and 1.90 mg/100 g zinc) (Kumar et al., 2019). The result also showed a higher concentration of minerals in the biscuit samples relative to GFM and GBGN flours (Table 1). This may be partly attributed to the disintegration of anti-nutritional compounds during baking resulting in the release of bound mineral elements. This is an indication of an improved micro-nutrient density of the biscuit samples. Minerals play significant roles in human physiology including the regulation of the immune system and heartbeat, the production of hormones and bone tissue, and the transmission of nerve impulses (Gharibzadeh and Jafari, 2017).

3.2. In vitro-protein digestibility (IVPD)

High IVPD (80.52–89.20 %) was recorded for the biscuit samples (Table 4). The digestibility of biscuits increased with an increasing level of GBGN. This may be ascribed to the high concentration of soluble globular proteins and amino acids in GBGN due to the modification of

Table 3. Mineral composition, total phenolic and antioxidant properties of biscuits.

Parameter	100GFM	90GFM: 10GBGN	80GFM: 20GBGN	70GFM: 30GBGN	60GFM: 40GBGN
Mineral composition					
Calcium (mg/100g)	188.45 ± 1.28 ^a	175.69 ± 1.11 ^b	170.50 ± 1.03 ^c	168.27 ± 1.51 ^d	165.88 ± 1.10 ^e
Iron (mg/100g)	273.69 ± 2.11 ^a	269.80 ± 1.94 ^b	266.33 ± 1.70 ^c	257.76 ± 1.45 ^d	252.60 ± 1.06 ^e
Magnesium (mg/100g)	1465.07 ± 5.26 ^a	1431.49 ± 3.30 ^b	1404.77 ± 1.93 ^c	1385.10 ± 2.01 ^d	1372.34 ± 1.88 ^e
Phosphorus (mg/100g)	2843.20 ± 2.51 ^a	2805.87 ± 1.92 ^b	2782.39 ± 2.10 ^c	2766.42 ± 1.53 ^d	2715.85 ± 2.06 ^e
Zinc (mg/100g)	5.95 ± 0.01 ^a	5.77 ± 0.01 ^a	5.61 ± 0.02 ^a	5.48 ± 0.01 ^a	5.32 ± 0.01 ^a
TPPC and antioxidant activity					
TPC (mg GAE/100 g)	189.11 ± 0.97 ^c	210.34 ± 1.10 ^d	235.27 ± 1.26 ^c	260.01 ± 1.29 ^b	273.42 ± 1.17 ^a
FRAP (μmol TE/100 g)	417.30 ± 1.20 ^c	432.83 ± 1.77 ^d	461.49 ± 1.10 ^c	490.25 ± 2.04 ^b	503.18 ± 1.92 ^a
ABTS (μmol TE/100 g)	212.78 ± 1.37 ^c	230.55 ± 1.12 ^d	240.29 ± 2.17 ^c	273.18 ± 1.24 ^b	296.10 ± 0.28 ^a

Mean and standard deviation of triplicates. Mean value with different superscript in a row are significantly ($p \leq 0.05$) different from each other. 100GFM = 100 % germinated finger millet; 90GFM:10GBGN = 90 % germinated finger millet and 10 % germinated Bambara groundnut flour; 80GFM:20GBGN = 80 % germinated finger millet and 20 % germinated Bambara groundnut flour; 70GFM: 30GBGN = 70 % germinated finger millet and 30 % germinated Bambara groundnut flour, 60GFM:40GBGN = 60 % germinated finger millet and 40 % germinated Bambara groundnut flour; TPC = Total phenolic content; FRAP = Ferric reducing antioxidant power; ABTS = 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) free radical scavenging assay.

Table 4. *In vitro* protein and starch digestibility of biscuits prepared from germinated finger millet and Bambara groundnut flour blends.

Parameter	100GFM	90GFM: 10GBGN	80GFM: 20GBGN	70GFM: 30GBGN	60GFM: 40GBGN
<i>In vitro</i> protein digestibility (%)	80.52 ± 0.81 ^c	82.06 ± 0.59 ^d	85.44 ± 0.90 ^c	86.93 ± 0.67 ^b	89.20 ± 0.73 ^a
Rapidly digestible starch (g/100 g)	20.44 ± 0.54 ^a	18.23 ± 0.26 ^b	15.60 ± 0.15 ^c	14.17 ± 0.24 ^d	12.56 ± 0.17 ^e
Slowly digestible starch (g/100 g)	69.85 ± 0.19 ^c	71.16 ± 0.34 ^d	72.85 ± 0.22 ^c	73.20 ± 0.13 ^b	74.39 ± 0.20 ^a
Resistant starch (g/100 g)	7.21 ± 0.27 ^c	7.95 ± 0.11 ^d	9.43 ± 0.19 ^c	12.61 ± 0.20 ^b	14.90 ± 0.16 ^a
C _∞ (g/100 g)	28.36 ± 0.23 ^a	27.22 ± 0.34 ^b	25.79 ± 0.20 ^c	24.50 ± 0.31 ^d	23.27 ± 0.24 ^e
k (min)	0.046 ± 0.01 ^a	0.033 ± 0.01 ^b	0.020 ± 0.00 ^c	0.017 ± 0.01 ^c	0.021 ± 0.01 ^c
Hydrolytic index (%)	33.68 ± 0.11 ^a	30.53 ± 0.19 ^b	28.61 ± 0.10 ^c	24.48 ± 0.13 ^d	21.17 ± 0.10 ^e
Glycemic index (%)	58.20 ± 0.15 ^a	56.47 ± 0.23 ^b	55.42 ± 0.27 ^c	53.15 ± 0.21 ^d	51.33 ± 0.19 ^e

Mean and standard deviation of triplicates. Mean value with different superscript in a row are significantly ($p < 0.05$) different from each other. 100GFM = 100 % germinated finger millet; 90GFM:10GBGN = 90 % germinated finger millet and 10 % germinated Bambara groundnut flour; 80GFM:20GBGN = 80 % germinated finger millet and 20 % germinated Bambara groundnut flour; 70GFM: 30GBGN = 70 % germinated finger millet and 30 % germinated Bambara groundnut flour, 60GFM:40GBGN = 60 % germinated finger millet and 40 % germinated Bambara groundnut flour C_∞: equilibrium concentration of starch hydrolyzed after 180 min, k: kinetic constant.

protein structures during the GBGN. Many factors may influence the digestibility of proteins in foods including the presence of dietary fiber, antinutritional factors and process variables among others. According to Chaitra et al. (2020), the inclusion of millet flour on wheat-based Belgian waffles decreased protein digestibility partly due to the repressing effect of both tannin and dietary fiber on protein digestibility.

3.3. *In vitro* starch digestibility and estimated glycemic index

The rapidly digested starch (RDS) content significantly decreased (20.44–12.56 g/100 g) while significant increase in slowly digested starch (SDS) (69.85–74.39 g/100 g) and resistant starch (RS) (7.21–14.90 g/100 g) were recorded with increasing proportion of GBGN (Table 4), which could be attributed to high fat, protein, dietary fiber and phenolic content of GBGN (Table 1). Starch can interact with various food components such as lipids, proteins, fiber, polyphenol amongst others, during processing and storage. Consequently, these food components inhibit enzymatic hydrolysis of starch by limiting the interaction or access between starch and enzymes resulting to low starch digestibility in food systems (Giuberti et al., 2018). In addition, the rate of starch digestion in foods is also influenced by extent of starch damage or gelatinization, size of the starch granules, composition and structure and physical encapsulation. The SDS, RDS and RS content of the biscuits obtained in this study agrees with the values obtained by Giuberti et al. (2018) who recorded percentage decreased contents of RDS (12.5–50.8%) and increased SDS (12.0–18.3%) as well as RS (2.7–9.3%) with increasing level of alfalfa seed flour in rice-based cookies. The RS content of biscuits produced from GFM and GBGN blends were higher compared to the value (6.34–7.73 g/100 g, dry weight) of cookie bar prepared from FM, kidney beans and arrowroot flour blends (Lestari et al., 2017). Rapidly digestible starch is fast digested and absorbed in the gastrointestinal tract and causes a rapid rise in blood sugar and insulin which may result to several health challenges such as cardiovascular diseases and diabetes after a long period of consumption (Xu et al., 2019). On the other hand, SDS offers a prolonged and sufficient release of glucose (Xu et al., 2019), while consumption of foods rich in RS could prevent colorectal cancer, lower plasma cholesterol and triglyceride level, inhibit fat accumulation, enhance hypoglycemic effect and micronutrient absorption (Raigond et al., 2015). This implies that the higher SDS and RS values of the biscuits are beneficial for consumers.

Parameters of *in vitro* starch digestibility of biscuits prepared from GFM-GBGN blends are presented in Table 4. The starch concentration at the equilibrium point (C_∞) and rate of hydrolysis of starch (k) were relatively low and decreased with increasing proportion of GBGN in the biscuit. Low value of C_∞ implied low digestible starch content while low k value denoted a slower digestion rate (Fering et al., 2016). This could indicate that the control (100% GFM) and GFM-GBGN biscuits contained low digestible starch with slower digestion rate. The hydrolysis index (HI)

and estimated glycemic index of the 100% GFM (control) biscuit was 33.68% and 58.20%, respectively, which are lower than the values (56.94 HI and 70.97 GI) reported for 100% wheat biscuit (Di Cairano et al., 2021). The HI and GI values of biscuits prepared from GFM-GBGN blends decreased (30.53–21.17% HI and 56.47–52.33 % GI) with increasing GBGN levels (Table 4), which may be attributed to low RDS, high SDS and RS. Low glycemic index is associated with low value of RDS, higher content of SDS and RS (Chaitra et al., 2020). In addition, the low GI of the biscuits may be due to high fiber content that caused decreased starch digestibility, and absorption of the carbohydrates (Maetens et al., 2017). The estimated GI of the GFM-GBGN biscuits blends were less than 60 and can be regarded as low GI products based on the global glycemic classification (GI < 60 is classified as low GI while GI > 60 as high) (Foster-Powell and Miller, 1995). Therefore, low GI of the formulated biscuits could contribute to control of diabetes, hyperlipidemia and obesity.

3.4. Antioxidant properties

The antioxidant properties, total phenolic content (TPC), ferric reducing antioxidant power (FRAP) and free radical scavenging ABTS values of GFM-based biscuit, increased significantly as the level of GBGN increased (Table 3). This could be connected to the higher antioxidant properties of GBGN (Table 1). The biscuit samples generally had a higher TPC and FRAP than GFM and GBGN flours, which could be due to the synthesis of phenolic compounds during baking (Mashau et al., 2020). This suggests a functionality enhancement in the composition of the biscuit samples. Increased antioxidant activity of the biscuit samples would help in counteracting the effect of free radicals and peroxides as well as promote the potency of anti-oxidative enzymes in the body (Pal et al., 2016). The TPC, FRAP and ABTS values of the 100% GFM and GFM-GBGN biscuits were relatively high compared to values reported for biscuits from acorn-hemp flour blends (Korus et al., 2017) and rice-alfalfa cookies (Giuberti et al., 2018).

3.5. Physical properties of biscuit

As observed from Table 5, most of the GFM-GBGN biscuits displayed similar diameter and thickness values. This is an indication that the biscuits have uniform rising ability during baking. The GFM-GBGN biscuits exhibited high spread ratio (SR) (ratio between the diameter and thickness) compared to the control, a desirable attribute in biscuit. The low SR recorded in GFM biscuit (control) may be attributed to high fiber content in the biscuit (Table 2) that caused reduction in SR, with a trend which is in agreement with the study of Di Cairano et al. (2021). There was no significant difference in SR among the composite biscuits, which aligns with the findings of Mancebo et al. (2016) who reported that increase in the pea protein concentration had no effect on the SR of

Table 5. Physical and sensory properties of biscuits prepared from germinated finger millet and Bambara groundnut flour blends.

Parameter	100GFM	90GFM: 10GBGN	80GFM: 20GBGN	70GFM: 30GBGN	60GFM: 40GBGN
Physical properties					
Thickness (cm)	1.15 ± 0.07 ^a	1.17 ± 0.04 ^a	1.12 ± 0.08 ^a	1.16 ± 0.05 ^a	1.14 ± 0.04 ^a
Diameter (cm)	4.39 ± 0.03 ^c	4.60 ± 0.01 ^a	4.54 ± 0.07 ^{ab}	4.65 ± 0.03 ^a	4.48 ± 0.05 ^b
Spread ratio	3.81 ± 0.01 ^b	3.93 ± 0.02 ^a	4.05 ± 0.05 ^a	4.01 ± 0.02 ^a	3.92 ± 0.01 ^a
Hardness (N)	10.52 ± 0.67 ^a	8.27 ± 0.20 ^b	7.83 ± 0.33 ^c	7.41 ± 0.65 ^c	7.10 ± 0.23 ^c
L*	36.70 ± 0.23 ^a	34.58 ± 0.16 ^b	33.14 ± 0.10 ^c	31.26 ± 0.18 ^d	30.49 ± 0.11 ^e
a*	4.11 ± 0.10 ^e	5.60 ± 0.14 ^d	6.19 ± 0.17 ^c	7.05 ± 0.11 ^b	8.23 ± 0.13 ^a
b*	16.08 ± 0.29 ^e	17.52 ± 0.23 ^d	19.66 ± 0.14 ^c	20.41 ± 0.19 ^b	22.56 ± 0.26 ^a
Sensory properties					
Appearance	7.25 ± 0.34 ^a	7.72 ± 0.60 ^a	7.89 ± 0.27 ^a	7.04 ± 0.61 ^b	7.14 ± 0.53 ^{ab}
Taste	7.40 ± 0.21 ^a	7.45 ± 0.19 ^a	7.56 ± 0.25 ^a	7.22 ± 0.51 ^{ab}	6.74 ± 0.29 ^b
Aroma	6.88 ± 0.20 ^a	6.84 ± 0.15 ^a	6.91 ± 0.48 ^a	6.53 ± 0.26 ^a	6.60 ± 0.22 ^a
Crunchiness	7.82 ± 0.19 ^a	7.60 ± 0.11 ^a	7.24 ± 0.16 ^a	6.85 ± 0.10 ^b	6.17 ± 0.21 ^c
Overall acceptability	7.49 ± 0.37 ^a	7.57 ± 0.20 ^a	8.03 ± 0.24 ^a	6.93 ± 0.28 ^b	6.22 ± 0.16 ^c

Mean value with different superscript in a row are significantly ($p \leq 0.05$) different from each other. 100GFM = 100 % germinated finger millet; 90GFM:10GBGN = 90 % germinated finger millet and 10 % germinated Bambara groundnut flour; 80GFM:20GBGN = 80 % germinated finger millet and 20 % germinated Bambara groundnut flour; 70GFM: 30GBGN = 70 % germinated finger millet and 30 % germinated Bambara groundnut flour, 60GFM:40GBGN = 60 % germinated finger millet and 40 % germinated Bambara groundnut flour. L* (lightness), a* (redness) and b* (yellowness).

rice-based cookies. Likewise, is also an agreement with the study of Adegbanke et al. (2019) which reported no significant difference in the spread ratio of cookies made from wheat flour and BGN, despite increasing levels of BGN substitution.

The textural (hardness) attribute of the samples (Table 5) showed that the biscuit produced from 100% GFM had the highest hardness (10.52 N). This could be due to the high carbohydrate content of GFM (Table 1). Mashau et al. (2020) had attributed the high hardness of tortilla prepared from 100 % maize flour to its high starch content and this led to the reduction in the water-holding capacity and increased rate of starch retro-gradation and shrinkage. The results also showed that the hardness of the biscuit reduced significantly with an increasing level of GBGN and the textural changes indicated softer texture of the biscuits. This could be due to the increasing level of protein and fat in the samples. The high protein content might have increased the rate of water absorption, thereby causing a reduction in rigidity (Mashau et al., 2020). Furthermore, the increased shortening effect of fat, in the blends of GFM and GBGN, might have been responsible for the reduction in hardness (Bolarinwa et al., 2019). The findings in this study agreed with that of Sibian and Riar (2020) who reported a decrease in the hardness of wheat-based cookie as the level of kidney bean (*Phaseolus vulgaris*) and chickpea (*Cicer arietinum*) increased. Yang et al. (2021) also recorded reduction in the hardness (15.2–13.2N) of cookies with increasing proportion of malted wheat flour in wheat-based cookies.

The L* value (36.70) was higher in control (100% GFM) biscuit compared to the GFM-GBGN biscuits (30.49–34.58). The a* and b* values of the composite biscuits increased ($p \leq 0.05$) with increasing proportions of GBGN in the composite biscuits. This may be ascribed to high content of protein and phenolics in the GBGN flour (Table 1). High contents of protein/amino acids in germinated flour are known to facilitate Maillard reaction during baking (Hnin et al., 2019) while increase in phenolic compounds facilitated melanoidin formation and caused darkening of the product during baking (Taranto et al., 2012). The L*, a* and b* values of the 100% GFM and GFM-GBGN biscuits align with the values reported for acorn-hemp biscuits (Korus et al., 2017).

3.6. Sensory properties of biscuits

Table 5 shows the sensory attributes of germinated finger millet and Bambara groundnut flour blend-based biscuits. There was no significant difference in consumer preference in terms of appearance, taste, crunchiness and overall acceptability for biscuits produced from 100%

GFM and the blends that contained 10 and 20% GBGN. The results showed higher consumer preference for these biscuit samples compared to the samples that contained ≥ 30 % of GBGN. Besides, no significant ($p \geq 0.05$) difference was recorded in terms of aroma among the biscuits. The high consumer preference, in terms of taste, appearance, and crunchiness, for the biscuit samples that contained 10 and 20% GBGN could be due to the dextrinization and browning reaction, of starch and protein molecules, which probably led to the elaboration of colour and flavour compounds (Sibian and Riar, 2020) as observed in the colour attributes of the samples (Table 5). The low acceptability of the biscuit samples that contained ≥ 30 % of GBGN could be due to the impartation of beany flavour, from the residual recalcitrant oligosaccharides in the biscuit. The sensory results showed good consumer acceptability of the biscuit produced from 100 % GFM. This is an indication of the good intrinsic physicochemical properties of germinated finger millet to produce value-added food products. As similar observation was reported in 100% malted finger millet biscuits with the study attributing this to the sweeter taste and better flavour of malted samples (Adebiji et al., 2017).

4. Conclusions

The blending of 80–90 % GFM with ≤ 20 % GBGN resulted in a biscuit with good nutritional, antioxidant, textural, and sensory qualities. The colour attributes of the biscuits were influenced by the addition of GBGN. Based on all the parameters investigated in this study, a combination of 80% germinated finger millet and 20 % GBGN composite biscuit will be best recommended. This is due to its comparable beneficial components, nutritional and sensory qualities, health promoting properties as well as low glycemic index, which all indicates its potential as a functional product. Nevertheless, further studies are required to evaluate the storage stability of the developed biscuits, structural elucidation of the products as well as a larger consumer acceptability test together with descriptive sensory analysis for better insights into the sensory characteristics and acceptability of the products.

Declarations

Author contribution statement

Chiemela Enyinnaya Chinma: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Patricia Ayuba Ibrahim; Olajide Emmanuel Adedeji; Vanessa Chinelo Ezeocha; Elizabeth Ugbede Ohuoba; Salamatu Ibrahim Kolo; Ruhaimat Abdulrahman: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nonyelum Laurentia Ogochukwu Anumba; Janet Adeyinka Adebo; Oluwafemi Ayodeji Adebo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2022.e10849>.

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