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Impact of germination alone or in combination with solid-state fermentation on the physicochemical, antioxidant, *in vitro* digestibility, functional and thermal properties of brown finger millet flours

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ABSTRACT

The impact of germination alone or in combination with solid-state fermentation on the physicochemical, antioxidant, *in vitro* digestibility, functional, and thermal characteristics of brown finger millet were evaluated. Germination and fermentation increased ($p \leq 0.05$) the protein, crude fiber, minerals, resistant starch (7.64–9.69 g/100g), total flavonoid content, total phenolic content, antioxidant properties (ABTS, DPPH and FRAP), majority of the amino acids as well as *in vitro* protein digestibility (67.72–89.53%), while antinutritional factors and digestible starch (from 45.17 to 35.58 g/100 g) content decreased significantly. Germination and fermentation significantly increased water absorption capacity and protein solubility, and slightly modified the pasting and thermal characteristics of brown finger millet flour while bulk density decreased. Among the treatments, combined germination and fermentation greatly improved the physicochemical, antioxidant, functional and processing properties of the flour with reduced antinutrients. Such combined process could enhance the use of brown finger millet as a novel flour in food product development.

1. Introduction

Finger millet (FM) is an underutilized whole grain cereal majorly grown in sub-Saharan Africa especially in Nigeria, and reported to have nutritional, nutraceutical and low-glycemic index advantages, but remains underutilized (Adebiyi, Obadina, Adebo, & Kayitesi, 2018; Jideani & Jideani, 2011). Finger millet is rich in essential amino acids such as histidine, lysine, methionine, tryptophan and also a good source of vitamins (Jideani, 2012; Saleh, Zhang, Chen, & Shen, 2013). However, the availability of these nutrients is limited by the presence of antinutritional factors (ANFs) especially phytic acid and oxalates inherent in the grains. Several processing techniques (such as roasting, soaking, cooking, germination fermentation), amongst others have been used to improve nutrient availability and subsequent products such as flour and starch from the grains have been used in the preparation of stiff porridge, weaning food, baked products and in pharmaceutical industries (Verma & Patel, 2013).

The primary objective of germination is to promote activation of inherent hydrolytic enzymes initially dormant in the raw seed (Ayernor & Ocloo, 2007) and has been known to improve nutritional value, cause structural modification, reduce ANFs, soften the kernel structure, and improve antioxidant and functional properties (Chinma, Abu, Asikwe, Sunday, & Adebo, 2021; Jimenez, Lobo, & Sammán, 2019; Kaukovirta-Norja, Wilhelmson, & Poutanen, 2004; Tian et al., 2010). Likewise is fermentation, a traditional method of processing plant foods that also decreases ANFs, enhances nutritional value, antioxidant, health

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beneficial, and functional properties of legumes and cereals (Adebiyi, Njobeh, & Kayitesi, 2019; Chinma et al., 2020).

According to the available literature, the combined impact of germination and solid-state fermentation (SSF) on the physicochemical, antioxidant activities, digestibility, functional and characteristics of brown finger millet is limited. It is thus important to understand the impact of these treatments on the composition of brown finger millet flours which may provide additional knowledge on the functionality of these grains. Such knowledge will contribute to increasing their utilization and potentials in the food industry, as well as contribute towards attaining food security in developing countries, where they are domesticated. The aim of this study was thus to determine the impact of germination alone or in combination with solid-state fermentation on the physicochemical, antioxidant, *in vitro* digestibility, functional, and thermal properties of brown finger millet flour.

2. Materials and methods

2.1. Materials

Finger millet (dark brown variety) grains and *Saccharomyces cerevisiae* (baker's yeast, Angel Yeast Company, Yichang Hubei, China) were procured from Central Market, Minna, Nigeria. All reagents used for the study are of analytical grade.

2.2. Sample preparation

Cleaned finger millet grains were washed, drained, and dried in air draft-oven (Gallenkamp, Cheshire, UK) at 40 °C for 24 h. The dried finger millet grains were milled and sieved (screen diameter 100 μm) to produce raw finger millet flour (RFMF), which served as control.

For the germination process, cleaned finger millet grains were sterilized with 0.07 g/L food grade sodium hypochlorite solution for 30 min, drained and then soaked (in distilled water) for 12 h at 28 ± 2 °C. The moistened FM grains were subsequently germinated at 25 °C for 48 h, and uniformly germinated grains were selected and dried for 24 h at 40 °C. Dried germinated finger millet grains were milled and sieved (100 μm mesh sieve) to obtain germinated finger millet flour (GFMF). The GFMF was divided into two portions. One portion was packed in polypropylene bags, kept in airtight container and stored at 4 °C prior to analyses, while the remaining portion was used for the subsequent preparation of another sample batch.

For the SSF process, fermented finger millet was prepared according to a standard procedure described by Ilowefah, Bakar, Ghazali, and Muhammad (2017). One gram (1 g) of dry yeast was mixed with 65 mL water and the suspension poured into 100 g RFMF and gently mixed for 2 min. The resulting mixture was covered with aluminium foil, and fermented at 27 °C, for 16 h in a fermentation cabinet (National MEG Company, Lincoln, USA). The fermented FM batter was oven dried for 24 h at 40 °C and the dried flour was blended and sieved (100 μm mesh sieve) to produce fermented finger millet flour (FFMF). For the germinated-fermented finger millet flour (GFFMF), 100 g of the GFMF was used instead of RFMF The batter was also dried, blended and sieved to obtain GFFMF.

2.3. pH and titratable acidity (TTA) determination

pH of the flours were analyzed by homogenizing 10 g of respective flour samples with 90 mL distilled water and determination using a calibrated pH meter (PHS-25, Techmel, USA). The filtered slurry of the sample was used to determine the TTA (%), which was titrated against 0.05 M NaOH solution with phenolphthalein as an indicator (AOAC, 2005).

2.4. Proximate analysis

Standard analytical procedures of the AOAC (2005) was used to determine: moisture (925.09), protein (total N × 6.25) (No. 992.23), ash (No. 923.03), fat (Soxhlet extraction method) (No. 920.39), ash (incineration in a muffle furnace for 24 h at 550 °C) (No. 923.03) and crude fiber (sample digestion with diluted acid and alkali) (No. 962.09) while total amylose content was measured following a standard method of Williams, Kuzina, & Hlynka (1971).

2.5. Resistant, digestible and total starch

A standard method (Englyst, Kingman, & Cummings, 1992) previously described by Chinma et al. (2021) was used in the analysis of resistant starch, and digestible starch by using megazyme kits (Megazyme Bray, Ireland), while starch (total) was obtained by addition of digestible starch and resistant starch (Chinma et al., 2021).

2.6. Phytic acid (PA) and oxalate content

The concentration of PA was measured following standard method by Latta and Eskin (1980) and involved extraction with HCl and passing the filtrate through AG1-X8 chloride anion exchange resin (Bio-Rad Laboratories GmbH, München, Germany). Thereafter, inorganic P (phosphorus) was eluted with 0.07 mol/L sodium chloride and the PA was determined colormetrically by absorbance (at 500 nm) on spectrophotometer (Genesys G10S, Thermo Fisher Scientific, Waltham, USA). Oxalate content was assayed according to the method of Ijarotimi (2008) by titrating the sample filtrate (after addition of H_2SO_4) against hot 0.1 mol/L $KMnO_4$ solution until a faint pink colour appeared and the oxalate content subsequently calculated.

2.7. Mineral analysis

Mineral composition [magnesium (Mg), calcium (Ca), zinc (Zn), potassium (K) and iron (Fe)] of the samples were performed using atomic absorption spectrophotometer (2380, PerkinElmer, Massachusetts, USA) according to method No. 985.35 of AOAC (2005). Phosphorus (P) was profiled using the flame photometric method (Method No. 984.27, AOAC, 2005). Briefly, 5 mL of the flour sample digest was pipette into a 50 mL volumetric flask and diluted to 50 mL with distilled water. The flame photometer was switched on and calibrated with standard solutions of phosphorus. Thereafter, an appropriate filter (photocell) was selected, and the atomizer of the instrument was dipped into the sample solution and the meter reading taken. The concentrations of the sample element were subsequently determined by extrapolating from the graph off the curve.

2.8. Amino acid (AA) profiling

Prior to analysis, each sample was hydrolyzed with 6 mol/L hydrochloric acid for 24 h at 116 °C and the amino acid composition was profiled using a HPLC (high-performance liquid chromatograph, PerkinElmer, Massachusetts, USA) with a C18 column (5 μm 100 × 3 mm) (Chrial Technologies, Munich, Germany) and detection with a photodiode array detector (MD-2010; JASCO, Tokyo, Japan) at 254 nm (Chinma, Ilowefah, Shammugasamy, Ramakrishnan, & Muhammad, 2014). The analytical standards were: alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine valine (Merck Pty, Johannesburg, South Africa). The mobile phase was a mixture of chromatographic grade acetonitrile, methanol and acetic acid (10:40:50, v/v/v).

2.9. In-vitro protein digestibility (IVPD)

Sample (200 mg of each flour) was weighed into 100 mL Erlenmeyer flask which contained 35 mL sodium citrate tribasic dihydrate (35 mL 0.1 mol/L, pH 2.0) with pepsin (1.5 g pepsin/L) (Ojokoh & Yimin, 2011). The resulting mixture was incubated for 2 h at 37 °C in a water bath (NLS42OS, Genlab Ltd., Cheshire, UK), centrifuged (K24IR Centurion Scientific Ltd, Chichester, UK) for 15 min at 10,000×*g*, and the supernatant decanted. Thereafter, the residue was washed, dried, and assayed for nitrogen content using a standard method (AOAC, 2005), and IVPD was calculated as the percentage of protein in supernatant divided by the total protein content of the sample.

2.10. Total flavonoid, total phenolic and antioxidant activity

The method of Chinma et al. (2014) was adopted for extraction using aqueous methanol (80%). The methanolic extracts were subsequently used for the total flavonoid, total phenolic and antioxidant activity assays. Total phenolic content (TPC) was measured following a standard procedure (Singleton & Rossi, 1965) in a spectrophotometer, and the results were defined on dry weight basis as mg of gallic acid equivalents/100g. Total flavonoid content (TFC) was measured colorimetrically as described by Bao, Cai, Sun, Wang, Corke, and (2005) as modified by Shen, Jin, Xiao, Lu, and Bao (2009). TFC was calculated using the standard rutin curve and results were expressed on dry weight basis as mg equivalents/100g. DPPH was obtained based on a standard method (Brand-Williams, Cuvelier, & Berset, 1995), absorbance measured at 516 nm DPPH was calculated using trolox as standard and results were defined as trolox equivalents (μmol TE/100g dry sample). FRAP was measured following a standard procedure of Queiros, Tafulo, and Sales (2013) and results expressed as trolox equivalents (μmol TE/100g dry sample). The ABTS radical cation activity was determined following a standard method (Awika, Rooney, Wu, Prior, & Zevallos, 2003) and results expressed as mg of trolox equivalents (TE)/100 g (dry basis).

2.11. Functional analysis

2.11.1. Bulk density (BD)

Bulk density of flour was measured following a standard method (Kaur & Singh, 2007). 1.5 g of sample was weighed into a graduated cylinder (10 mL) and the cylinder was gently tapped until there was no further reduction in the sample level. BD (g/cm^3) was defined as the weight of sample/volume of sample.

2.11.2. Oil and water absorption capacity

Water and oil absorption capacity (WAC and OAC) was measured according to standard method (AACC method 56–20, 2000). For WAC, distilled water (10 mL) was added to 1 g of sample in a weighed centrifuge tube, mixed and kept for 30 min at 28 °C. Thereafter, the resulting mixture was centrifuged for 10 min at 2000×*g* and the supernatant decanted. For OAC, refined soybean oil substituted water. OAC and WAC were measured as the difference between the weights (initial and final) after the oil/water added had been removed.

2.11.3. Swelling power (SP)

For the SP, flour was filled up to the 10 mL mark in a graduated cylinder (100 mL) after which distilled water was carefully added to bring the volume to 50 mL. Subsequently, the graduated cylinder was tightly covered, and mixed by gently inverting the cylinder and after 2 min, allowed to stand for 30 min. The volume occupied by the flour (after 30 min) was read as computed (Okaka & Potter, 1977).

2.12. Pasting profile and thermal characteristics

Pasting parameters (break down, final, trough, setback and peak viscosities as well as pasting temperature and peak time) were obtained using a rapid visco analyzer (RVA, Newport 8 Scientific Pty Ltd., New South Wales 2102, Australia) (Chinma et al., 2016). To 2.5 g of respective flour samples, 25 mL of distilled water was added and theresulting suspension was mixed and placed in the RVA. The temperature program were as follows: initially kept at 50 °C for 1 min, then heated to 95 °C at 12.2 °C/min and held at 95 °C for 2.5 min. This was later cooled to 50 °C for 2 min at the rate of 11.8 °C/min.

A differential scanning calorimeter (DSC) (Model 204, Nietzsche, Germany) was used to measure thermal parameters (peak, onset, and conclusion temperature, and gelatinization enthalpy (ΔH), (Chinma, Anuonye, Simon, Ohiare, & Danbaba, 2015). The flours (5 mg) were mixed with distilled water (1:3, w/w) and thermal analyses conducted from 25 °C to 120 °C at a rate of 10 °C/min.

2.13. Statistical analysis

All analyses were measured in triplicates and data obtained subjected to analysis of variance using SPSS 22 (IBM, Armonk, USA). Differences among the means of the measured parameters were separated by Tukey's test at 5% probability.

3. Results and discussion

3.1. pH and TTA

The pH of raw finger millet was 6 (Table 1), which is close to the value (6.32) reported by Mutshinyani, Mashau, and Jideani (2020) for raw finger millet (dark brown variety). The pH of germinated finger millet flour (GFMF), fermented finger millet flour (FFMF), and germinated-fermented finger millet flour (GFFMF) samples were reduced by 9.09%, 10.61% and 12.12%, respectively, compared to the raw finger millet. On the other hand, TTA of germinated, fermented, and germinated-fermented FM flour significantly increased by 9%, 4% and 4%, respectively compared to raw finger millet. The reduction in pH values of the treated FM samples could be due to breakdown of complex organic molecules by microorganisms that led to accumulation of organic acids, which increased the acidity. Similar trends were observed by other authors (Adebo, Njobeh, Adebiyi, & Kayitesi, 2018; Chinma et al., 2020; Siddiqua, Ali, & Ahmed, 2019) for pH and TTA of germinated and fermented grains.

3.2. Proximate composition

The proximate content of raw finger millet (Table 1) is in close range with values previously reported by Mutshinyani et al. (2020) for dark brown variety of finger millet. The moisture value of samples were ≤10% recommended as safe limit for extended preservation of flours. Raw FM contained 9.03 g/100g protein, which increased by 14.73%, 19.27% and 19.60% in germinated, fermented and germinated-fermented finger millet flours, respectively. The increased concentration of protein in the bioprocessed finger millet flour samples could be attributed to synthesis of enzymes by the fermenting or germinating grains, synthesis of newly formed proteins, and degradation of other constituents such as antinutritional factors (ANFs) (Ilowefah et al., 2017; Ohanenye, Tsopmo, Ejike, & Udenigwe, 2020; Xu et al., 2019). Owheruo, Ifesan, and Kolawole (2019) recorded slight increase in protein value (7.61–7.81 g/100 g) of finger millet after 72 h germination. In contrast, reduction in protein content of finger millet after natural fermentation (8–36 h) has also been reported (Narayanasamy, 2020). Raw finger millet contained 1.82 g/100 g fat, which decreased by 21.4%, 39.34% and 38.35% after germination, fermentation, combined germination and fermentation processes, respectively. This could be attributed to lipolytic hydrolysis caused by lipase enzyme during germination/fermentation (Adebiyi et al., 2019) or the utilization of lipids as energy sources during solid-state fermentation and/or germination. Ash value of raw finger millet was 2.44 g/100g, and increased by

Table 1
Chemical composition, ANFs, TPC and antioxidant activities of raw, germinated, fermented, germinated and fermented brown finger millet flours.

Parameters	Raw flour	Germinated FM	Fermented FM	Germinated and fermented FM
pH, TTA and proximate composition				
pH	6.60 ± 0.01^{a}	6.00 ± 0.00^{b}	5.90 ± 0.01^{b}	5.80 ± 0.01^{b}
Titratable acidity (%)	0.10 ± 0.00^{b}	0.19 ± 0.01^{a}	0.14 ± 0.01^{a}	0.14 ± 0.06^{a}
Moisture (g/100 g)	8.15 ± 0.06^{a}	8.37 ± 0.10^{a}	8.42 ± 0.07^{a}	8.17 ± 0.04^{a}
Protein (g/100 g)	9.03 ± 0.40^{c}	10.36 ± 0.21^{b}	10.77 ± 0.14^{a}	10.80 ± 0.28^{a}
Fat (g/100 g)	1.82 ± 0.01^{a}	1.43 ± 0.04^{b}	1.10 ± 0.02^{c}	1.13 ± 0.01^{c}
Ash (g/100 g)	2.44 ± 0.12^{b}	2.96 ± 0.10^{a}	2.37 ± 0.11^{b}	2.80 ± 0.10^{a}
Crude fiber (g/100 g)	3.86 ± 0.23^{b}	4.25 ± 0.16^{a}	3.44 ± 0.26^{c}	4.11 ± 0.22^{a}
Starch characteristics				
Amylose (g/100 g)	22.87 ± 0.72^{a}	21.70 ± 0.66^{b}	20.34 ± 0.57^{c}	19.10 ± 0.57^{d}
Total starch (g/100 g)	52.71 ± 0.20^{a}	49.50 ± 0.35^{b}	48.92 ± 0.22^{c}	45.26 ± 0.11^{d}
Resistant starch (g/100 g)	7.54 ± 0.05^{a}	8.62 ± 0.01^{b}	9.10 ± 0.14^{c}	9.69 ± 0.20^{d}
Digestible starch (g/100 g)	45.17 ± 0.11^{a}	40.88 ± 0.26^{b}	39.82 ± 0.35^{c}	35.58 ± 0.51^{d}
ANFs				
Phytic (mg/100 g)	65.80 ± 0.44^{a}	32.17 ± 0.56^{b}	28.54 ± 0.70^{c}	26.60 ± 0.40^{d}
Oxalate (mg/100 g)	5.16 ± 0.18^{a}	2.35 ± 0.22^{b}	1.67 ± 0.19^{c}	1.44 ± 0.13^{d}
Mineral composition				
Calcium (mg/100 g)	124 ± 0.16^{d}	147 ± 0.10^{a}	135 ± 0.22^{c}	136 ± 0.22^{b}
Iron (mg/100 g)	181 ± 0.75^{a}	406 ± 0.42^{a}	394 ± 0.86^{a}	401 ± 0.93^{a}
Magnesium (mg/100 g)	1095 ± 1.68^{d}	1265 ± 2.06^{a}	1120 ± 1.15^{c}	1189 ± 1.46^{b}
Potassium (mg/100 g)	2120 ± 1.24^{d}	2208 ± 1.63^{a}	2165 ± 0.93^{c}	2194 ± 1.28^{b}
Phosphorus (mg/100 g)	2278 ± 1.19^{d}	2495 ± 1.85^{a}	2403 ± 1.14^{c}	2452 ± 2.03^{b}
Zinc (mg/100 g)	16.10 ± 0.44^{d}	18.69 ± 0.50^{a}	17.44 ± 0.29^{c}	18.20 ± 0.16^{b}
Total phenolic and total flavonoid content, PC and antioxidant activity				
TPC (mg/GAE/100 g)	122 ± 0.03^{d}	140 ± 0.01^{c}	155 ± 0.04^{b}	161 ± 0.07^{a}
TFC (mg RE/100 g)	119 ± 0.85^{d}	135 ± 0.62^{c}	143 ± 0.55^{b}	155 ± 0.48^{a}
DPPH (μmolTE/100 g)	131 ± 0.36^{d}	142 ± 0.27^{c}	154 ± 0.76^{b}	169 ± 0.45^{a}
FRAP (μmol TE/100 g)	120 ± 0.13^{d}	138 ± 0.10^{c}	147 ± 0.08^{b}	159 ± 0.11^{a}
ABTS radical scavenging ability (%)	36.80 ± 0.24^{d}	51.94 ± 0.33^{c}	72.10 ± 0.27^{b}	78.55 ± 0.46^{a}

Mean values and standard deviation of triplicate replications. Means with no common letters within a row differed ($p \leq 0.05$). ABTS - 2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid; ANFs - Antinutritional factors; DPPH - 1,1-diphenyl-2-picryl-hydrazil radical scavenging activity; FRAP - ferric reducing antioxidant power; FM – finger millet; TTA – titratable acidity; TPC – total phenolic content; TFC – total flavonoid content.

21.31% and 14.75% after germination, and germination and fermentation, respectively, while a reduction in ash value (28.70%) was recorded after fermentation of FM which could be due to from increased loss of dry matter due to enzyme activities and yeast proliferation during fermentation (Chinma et al., 2020). Crude fiber content increased by 10.10% and 6.48% in germinated, and germinated-fermented FM flour, respectively, compared to the control, whereas a reduction (10.88%) in crude fiber value was recorded in fermented sample. The increase in crude fiber content in germinated FM may stem from formation of new primary cell whereas the observed reduction in crude fiber value after fermentation could partly be attributed to degradation of crude fiber by enzymes (Chinma et al., 2021; Ilowefah et al., 2017).

3.3. Starch characteristics

Table 2 shows the starch characteristics of raw (RFMF), germinated (GFMF), fermented (FFMF) and germinated-fermented (GFFMF) finger millet flours. Amylose, total starch (TS) and digestible starch (DS) of native finger millet flour decreased with germination, fermentation, and combination of germination-fermentation, while the resistant starch increased for the same treatments. Amylose content of GFMF, FFMF and GFFMF decreased by 5.12%, 11.06% and 16.48%, respectively, compared to the control. Amylose content of raw and processed FM flours are within the reported range (20–34%) for different species of millet (Annor, Marcone, Bertoft, & Seetharaman, 2014; Hoover, Swamidas, Kok, & Vasanthan, 1996). Li, Jeong, Lee, and Chung (2020) recorded reduction in apparent amylose content of millet and sorghum after germination (24–48 h), and ascribed the reduction to degradation of amylose by enzymes stimulated by the germination process. Similar effects on amylose have also been reported after fermentation of sorghum flour (Afify, El-Beltagi, Abd El-Salam, & Omran, 2012).

Total starch levels of raw finger millet decreased in GFMF, FFMF and GFFMF by 6.1%, 7.2% and 14.13%, respectively. Starch reduction from 71.3% to 35.1% during germination (0–96 h) of finger millet has been reported (Mbithi-Mwikya, Van Camp, Yiru, & Huyghebaert, 2000), with

Table 2
Amino acid composition (g/100 g) and *in vitro* protein digestibility of raw, germinated, fermented and germinated-fermented brown finger millet flours.

Parameters	Raw flour	Germinated flour	Fermented flour	Germinated-fermented flour
Essential amino acids				
Histidine	0.26 ± 0.01^{a}	0.23 ± 0.01^{a}	0.25 ± 0.01^{a}	0.26 ± 0.01^{a}
Isoleucine	0.38 ± 0.02^{b}	0.46 ± 0.01^{a}	0.47 ± 0.01^{a}	0.47 ± 0.00^{a}
Leucine	0.69 ± 0.04^{b}	0.74 ± 0.02^{a}	0.76 ± 0.03^{a}	0.77 ± 0.01^{a}
Lysine	0.25 ± 0.00^{a}	0.37 ± 0.01^{a}	0.38 ± 0.00^{a}	0.38 ± 0.01^{a}
Methionine	0.21 ± 0.01^{b}	0.30 ± 0.00^{a}	0.30 ± 0.01^{a}	0.30 ± 0.00^{a}
Phenylalanine	0.33 ± 0.02^{b}	0.42 ± 0.01^{a}	0.43 ± 0.00^{a}	0.43 ± 0.01^{a}
Threonine	0.28 ± 0.01^{b}	0.34 ± 0.01^{a}	0.36 ± 0.03^{a}	0.37 ± 0.01^{a}
Valine	0.48 ± 0.02^{b}	0.51 ± 0.04^{a}	0.52 ± 0.01^{a}	0.53 ± 0.02^{a}
Non-essential amino acid				
Alanine	0.51 ± 0.01^{b}	0.63 ± 0.04^{a}	0.65 ± 0.03^{a}	0.67 ± 0.01^{a}
Arginine	0.39 ± 0.00^{b}	0.50 ± 0.01^{a}	0.52 ± 0.01^{a}	0.54 ± 0.03^{a}
Aspartic acid	0.53 ± 0.01^{a}	0.57 ± 0.02^{a}	0.58 ± 0.03^{a}	0.57 ± 0.02^{a}
Cysteine	0.13 ± 0.02^{a}	0.14 ± 0.01^{a}	0.15 ± 0.02^{a}	0.16 ± 0.01^{a}
Glutamic acid	1.21 ± 0.03^{b}	1.33 ± 0.05^{a}	1.34 ± 0.03^{a}	1.34 ± 0.04^{a}
Glycine	0.24 ± 0.01^{a}	0.27 ± 0.01^{a}	0.27 ± 0.01^{a}	0.27 ± 0.01^{a}
Proline	0.47 ± 0.02^{b}	0.52 ± 0.04^{a}	0.56 ± 0.02^{a}	0.55 ± 0.04^{a}
Serine	0.40 ± 0.01^{a}	0.43 ± 0.01^{a}	0.42 ± 0.01^{a}	0.43 ± 0.01^{a}
Tyrosine	0.25 ± 0.00^{b}	0.34 ± 0.02^{a}	0.38 ± 0.03^{a}	0.38 ± 0.02^{a}
In vitro protein digestibility (%)	67.72 ± 0.81^{d}	80.16 ± 0.95^{c}	82.40 ± 0.66^{b}	89.53 ± 0.74^{a}

Mean values and standard deviation of triplicate replications. Means with no common letters within a row differed ($p \leq 0.05$).

a corresponding increase in sugars signifying starch hydrolysis by amylolytic enzymes. The 7.2% starch reduction in FFMF obtained in this study is similar to 7.4% starch reduction reported by Usha, Sripriya, and Chandra (1996) during 48 h fermentation of finger millet flour, similar results are also reported after fermentation of foxtail and pearl millet (Antony, Sripriya, & Chandra, 1996; Khetarpaul & Chauhan, 1990). Reduction in starch after fermentation indicates starch hydrolysis by the fermenting microbes which is also evident by the reported marked increase in soluble and reducing sugars following fermentation (for 48 h) (Usha et al., 1996).

Digestible starch of GFMF, FFMF and GFFMF decreased by 9.5%, 11.84%, and 21.25%, respectively, when compared with the control, while resistant starch increased by 14.32%, 20.69%, and 28.51% for GFMF, FFMF and GFFMF, respectively (Table 1). Amadou, Gounga, and Le (2013) reported an increase in resistant starch (RS), rapidly digestible starch (RDS) and slowly digestible starch (SDS) of fermented foxtail millet flour, with a more pronounced increase for SDS and RS compared to RDS. On the contrary, other studies reported an increase in digestible starch and a decrease in resistant starch in germinated millet and other legumes (Benítez et al., 2013; Roopa & Premavalli, 2008). The impact of germination and fermentation on these starch fractions is influenced by amylase activity and presence of ANFs. Germination and fermentation promote endogenous α-amylase activity and reduce phytates that inhibit amylase activity thus, increasing starch hydrolysis and digestibility. In this study, phytic acid and oxalate decreased significantly in GFMF, FFMF and GFFMF (section 3.4). Overall, changes in starch characteristics of raw FMF were similar in GFMF and FFMF, while changes were larger in GFFMF when compared to GFMF and FFMF. These results indicate that greater effects on physicochemical modification can be obtained with combined processing strategies such as germination and fermentation.

3.4. Antinutritional factors (ANFs)

Phytic acid (PA) and oxalate contents of the native FM, GFMF, FFMF and GFFMF samples are shown in Table 1. The PA content of raw brown finger millet recorded in this study is lower compared to the value recorded by Nakarani et al. (2021) for some finger millet genotypes from India. The initial concentration (65.80 mg/100 g) of PA in raw finger millet decreased by 51.11%, 56.63% and 59.57% after germination, fermentation, and combined germination and fermentation treatments, respectively. The decrease in PA could be ascribed to the effect of germination and or solid-state fermentation which increased the activity of inherent or native phytase that can hydrolyze insoluble organic complexes with minerals (Olukomaiya et al., 2020). Owheruo et al. (2019) recorded 23.54% reduction in PA after 72 h germination of finger millet (cream variety). Shimelis and Rakshit (2007) also reported decrease in PA content after germination and fermentation of grains.

Oxalate content of raw finger millet was 5.16 mg/100 g which is within the range observed for other varieties of native finger millet (Ravindran, 1991). Oxalate content of raw FM significantly decreased by 54.46%, 67.67% and 72.09%, after germination, fermentation, and combined germination and fermentation treatment, respectively. Reduction in oxalate value may be ascribed to leaching of oxalate during soaking of finger millet grains prior to germination and/or fermentation. The reduction of oxalate in food is important because oxalate interferes with calcium availability in the body. The combination of germination and fermentation had the highest effect on ANFs reduction in finger millet, followed by fermentation, and germination.

3.5. Mineral composition

Mineral content of the of the native FM, GFMF, FFMF and GFFMF samples is shown in Table 1. The Ca, Fe, Mg, K, P and Zn contents of raw finger millet were 124 mg/100 g, 181 mg/100 g, 1095 mg/100 g, 2120 mg/100 g, 2278 mg/100 g and 16.10 mg/100 g, respectively, which is comparable to the values (130 mg/100g Ca, 178 mg/100 g Fe, 1119 mg/100 g Mg and 2686 mg/100 g P) reported for raw pearl millet (Obadina et al., 2016). The Ca, Fe, Mg, K, P and Zn contents of FM increased by 18.30%, 124%, 15.55%, 4.11%, 9.56% and 16.09%, respectively, after germination. Likewise, Ca, Fe, Mg, K, P and Zn content of finger millet increased by 8.76%, 117%, 2.33%, 2.09%, 5.51% and 8.32%, respectively after fermentation. Germination and fermentation also increased the Ca, Fe, Mg, K, P and Zn contents by 9.77%, 121%, 8.62%, 3.49%, 7.65% and 13.04%, respectively, compared to raw finger millet. The mineral results revealed that germination process was effective in enhancing the mineral composition of brown finger millet flour, followed by a combination of germination and fermentation processes. The significant increase ($p \leq 0.05$) in mineral value of the bioprocessed finger millet samples could be ascribed to reduction in ANFs during germination and or/fermentation (El-Adawy, Rahma, El-Bedawey, & El-Beltagy, 2003; Nkhata, Ayua, Kamau, & Shingiro, 2018). The increase in mineral content of the processed finger millet flours are vital for provision of these nutrients for normal body functions.

3.6. Total phenols, total flavonoid and antioxidant activity

The TPC, TFC and antioxidant activity (ABTS, DPPH and FRAP assays) of the raw and processed flour samples are presented in Table 1. TPC, TFC, DPPH, FRAP and ABTS scavenging activities of raw finger millet were 1.22 mg/GAE/100 g, 119 mg/RE/100 g, 131 μmolTE/100 g, 1.20 μmolTE/100 g and 36.80%, respectively. Percentage increases in TPC, TFC, DPPH, FRAP and ABTS after germination were 16.67%, 13.62%, 8.98%, 13.11% and 51.94%, respectively, while percentage increases in TPC, TFC, DPPH, FRAP and ABTS after fermentation were 27.05%, 20.40%, 18.31%, 20.49% and 72.10%, respectively. Similarly, combined germination and fermentation of finger millet increased TPC, TFC, DPPH, FRAP and ABTS by 31.97%, 30.86%, 29.24%, 30.33% and 78.55%, respectively. The observed increase in total phenolics in GFMF could be ascribed to activation of enzymes that facilitate the formation of phenolic compounds (Salawu, Bester, & Duodu, 2014). In another study, it was reported that germination of rye grains at different temperature for 6 days increased the methanol extractable phenolic compounds and was associated with synthesis of hydrolytic enzymes resulting in modification of cell-wall structure, and synthesis of new compounds with bioactive potentials (Liukkonen et al., 2003). Therefore, increase in antioxidant activities (TFC, DPPH, FRAP and ABTS) of FM after germination may be associated with increase in phenolic compounds (Ferreira et al., 2019). Similarly, fermentation has been reported to increase TPC and antioxidant activities in cereals which could be attributed to the activities of enzymes produced by microbial activities, metabolism of phenolic compounds by fermenting microorganisms and the release of previously bound phenols (Adebo & Medina-Meza, 2020).

3.7. Amino acid composition and in vitro protein digestibility (IVPD)

The amino acid composition (AAs) of RFMF, GFMF, SFFMF and GSFFMF are shown in Table 2. Generally, both fermentation and germination processes increased the amino acid contents. The highest content of essential AAs was leucine (0.69–0.77 g/100 g) while the highest concentration of a non-essential AA was glutamic acid (1.21–1.34 g/100 g). The total non-essential AAs in RFMF increased by 17.45, 21.14 and 22.48% for GFMF, SFFMF and GSFFMF, respectively, while total essential AAs in RFMF increased by 14.53, 17.92 and 18.89% in GFMF, SFFMF and GSFMFF, respectively. Research studies have associated the increase in protein content and AAs of sprouted plant to degradation of nutrients such as carbohydrate and fat in the synthesis of protein; and reduction in ANFs previously bound to the AAs (Sade, 2009). Increase in AAs content after fermentation has been previously attributed to the increase protein hydrolysis and microbial enzyme activities during fermentation (Sripriya, Antony, & Chandra, 1997).

The IVPD of RFMF, GFMF, SFFMF and GSFFMF shown in Table 2. IVPD of RFMF was 67.72% which is within the IVPD range (55.4%–88.1% reported for some finger millet varieties (Ramachandra, Virupaksha, & Shadaksharaswamy, 1977). IVPD of raw FM (67.72%) increased to 80.16% after germination, 82.40% after fermentation, and 89.53% following combined germination and germination. Differences in IVPD between germinated and fermented finger millet flour were minimal, while a combination of both treatments had larger effects. Improved IVPD in germinated pearl and finger millet flour has been previously reported (Hejazi & Orsat, 2016; Khetarpaul & Chauhan, 1990; Mbithi-Mwikya et al., 2000; Pushparaj & Urooj, 2011). During germination, hydrolytic enzymes breakdown proteins into smaller units increasing their bioavailability and digestibility (Singh, Rehal, Kaur, & Jyot, 2015). The storage proteins are hydrolyzed by endogenous proteases making them readily available for pepsin digestion (Pushparaj & Urooj, 2011). Increased proteolysis and partial solubilization evidenced by increased levels of amino acids during germination was also reported to be responsible for improved IVPD in germinated millet flour (Mbithi-Mwikya et al., 2000). Similar results have also been reported for fermented pearl and finger millet flour (Ali, El Tinay, & Abdalla, 2003). Significant increase in IVPD was reported by Ali et al. (2003) after 14 h natural fermentation of two cultivars of pearl millet and the IVPD values were similar to results obtained by Elyas, El Tinay, Yousif, and Elsheikh (2002) after 36 h natural fermentation. Increase in IVPD during fermentation is ascribed to increased proteolytic enzyme activity, leading to the degradation of complex proteins to smaller and soluble ones (Rathore, Singh, Kamble, Upadhyay, & Thangalakshmi, 2019). Increased IVPD of pearl millet after combined treatment of germination and fermentation has been shown in previous studies with a combination of these processes causing significant improvement in IVPD values (Hassan et al., 2006), although minimal increases were observed in the study of Onyango et al. (2013). Reduction in ANFs also contributes to increased protein digestibility in millet and other cereal grains because their presence inhibits proteolytic activity as they can bind with protein molecules (Becker & Yu, 2013). IVPD results in this study show that germination and fermentation are effective strategies that can be employed to improve protein digestibility in brown finger millet with a combination of both treatments providing greater effects than the individual treatments alone.

3.8. Functional properties

Table 3 shows the functional properties of raw, germinated, fermented, and germinated-fermented finger millet. Bulk density (BD) of raw finger millet decreased by 44.21%, 57.89% and 56.84% after germination, fermentation, and combined germination and fermentation, respectively. Reduction in BD after malting/germination and fermentation have been ascribed to the breakdown of complex denser carbohydrates and proteins into smaller ones that are less bulky (Adebiyi, Obadina, Mulaba-Bafubiandi, Adebo, & Kayitesi, 2016). The reduced BD of the bioprocessed brown finger millet flours would find useful application in food formulations where low bulk density is required.

Water absorption capacity (WAC) of raw brown finger increased by 33.24%, 67.04% and 117.65% after germination, fermentation and combined germination and fermentation, respectively. Elkhalifa and Bernhardt (2010) suggested that the increase in WAC could be due to increased protein value, and changes in protein quality during germination alongside the breakdown of polysaccharide molecules. The high WAC of the bioprocessed brown finger millet flours suggests their potential application in confectionaries where hydration is essential to the food properties.

Swelling power (SP) of raw FM (8.69%) decreased significantly after germination (8.10%), fermentation (7.44%), and combined germination and fermentation (7.02%), which may stem from decrease in starch content and changes in starch structure caused by activities of enzymes during germination and/or fermentation (Chinma et al., 2015; Ilowefah et al., 2017). This observation could be justified by the fact that starch is a major constituent of cereal flour, and its structure influences functional properties of the flour such as swelling power (Wang, Wang, Li, Wei, & Adhikari, 2012). Similar SP results has been reported after germination, and fermentation of rice (Chinma et al., 2015; Ilowefah et al., 2017).

Protein solubility (PS) of raw finger millet was 37.45%, which increased by 66.30%, 72.74% and 81.84% after germination, fermentation, and combined germination and fermentation, respectively. Similar increase in PS after germination (10–48 h) has been reported in sorghum (40.25–84.95%) (Singh, Sharma, & Singh, 2017). Such increase in PS has been attributed to protein degradation into peptides and free amino acids leading to increased solubility (Singh & Sharma, 2017).

Table 3
Functional and thermal properties of raw, germinated, fermented and germinated-fermented brown finger millet flours.

Parameters	Raw flour	Germinated flour	Fermented flour	Germinated-fermented flour
Functional properties				
Bulk density (g/cm^3)	0.95 ± 0.01^a	0.53 ± 0.01^b	0.40 ± 0.07^c	0.41 ± 0.01^c
Water absorption capacity (g/g)	3.58 ± 0.03^d	4.77 ± 0.01^c	5.98 ± 0.02^b	6.36 ± 0.06^a
Oil absorption capacity (g/g)	1.33 ± 0.01^a	1.21 ± 0.04^a	1.12 ± 0.02^a	1.14 ± 0.01^a
Swelling power (%)	8.69 ± 0.14^a	8.10 ± 0.22^b	7.44 ± 0.16^c	7.02 ± 0.10^d
Protein solubility (%)	37.45 ± 0.10^d	62.28 ± 0.17^c	64.69 ± 0.35^b	68.10 ± 0.14^a
Pasting properties				
Peak viscosity (cP)	2456 ± 2.89^a	654 ± 1.96^c	935 ± 1.11^b	446.00 ± 1.03^d
Trough viscosity (cP)	2029 ± 1.75 ^{a}	322 ± 1.51^d	860 ± 1.24^b	423 ± 2.71^c
Break down (cP)	427 ± 1.20^a	332 ± 1.10^b	75 ± 1.05^d	23 ± 1.16^c
Final viscosity (cP)	2742 ± 2.30^a	612 ± 1.38^d	1102 ± 1.07^b	892 ± 1.65^c
Setback viscosity (cP)	713 ± 1.26^a	290 ± 1.86^c	242 ± 1.15^d	469 ± 1.34^b
Pasting-temperature (^{o}C)	76.70 ± 0.30^d	79.15 ± 0.51^c	92.95 ± 0.69^a	85.70 ± 0.48^b
Peak time (Min)	6.07 ± 0.12^b	4.93 ± 0.17^c	6.80 ± 0.14^a	7.00 ± 0.21^a
Thermal properties				
Onset temperature (^{o}C)	64.22 ± 0.53^d	66.31 ± 0.44^c	69.39 ± 0.23^b	70.52 ± 0.46^a
Peak temperature (^{o}C)	75.60 ± 0.44^d	78.82 ± 0.32^c	80.15 ± 0.77^b	81.23 ± 0.50^a
Conclusion temperature (^{o}C)	79.14 ± 0.26^d	81.67 ± 0.57^c	83.22 ± 0.46^b	85.17 ± 0.44^a
Enthalpy of gelatinization (J/g)	6.85 ± 0.14^a	6.30 ± 0.29^b	5.75 ± 0.14^c	5.02 ± 0.10^d

Mean values and standard deviation of triplicate replications. Means with no common letters within a row significantly differ ($p \leq 0.05$).

3.9. Pasting properties

The pasting profiles of the raw and bioprocessed brown finger millet flour samples are presented in Table 3 and Fig. 1. Significant reduction in pasting viscosities of brown finger millet flours were recorded in the bioprocessed finger millet compared to raw flour, while pasting temperature increased (76.70–92.95 °C). Peak and final viscosities of bioprocessed brown FM flours were in this order: fermented > germinated > germinated-fermented. On the other hand, germinated-fermented finger millet flour had the lowest break down viscosity (23 ± 1.16 cP), followed by fermented FM (75 ± 1.05 cP), indicating strong shearing

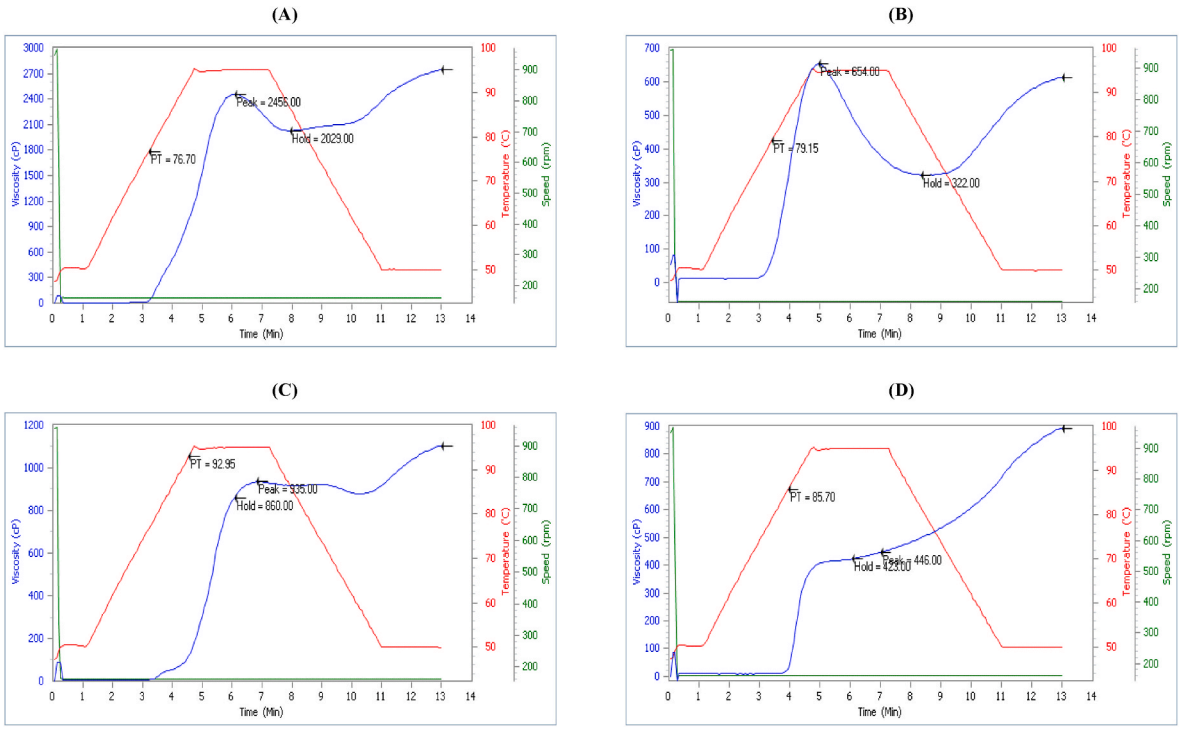

Fig. 1. Typical pasting profiles of raw finger millet flour (A), germinated finger millet flour (B), solid-state fermented finger millet (C) and germinated-solid state fermented finger millet flour (D).

resistance and good paste stability (Wani, Andrabi, Sogi, & Hassan, 2020). Similar reduction of viscosity parameters were reported in malted pearl millet (Obadina et al., 2016) and fermented koreeb seed flour (Ahmed, Sulieman, et al., 2019). The reduction in pasting viscosities of the bioprocessed finger millet flour samples may partly be ascribed to degradation of starch by α-amylase activity, and protein hydrolysis by protease (Xu et al., 2017). It has been reported that protease and α-amylase activity increases rapidly during germination, and fermentation (Abd et al., 2005; Cornejo, Caceres, Martínez-Villaluenga, Rosell, & Frias, 2015) and such in reflected in the degradation of inherent components. Higher pasting temperature of the bioprocessed finger millet flour samples than raw flour is ideal for thickening food or food requiring high gel strength (Xu et al., 2019). Pasting time of the native and germinated flours differed significantly, with germinated finger millet flour having the lowest pasting time of 4.93 min. This suggests that the germinated finger millet flour requires lesser time to cook than the raw, fermented, and germinated and fermented brown finger millet flour.

3.10. Thermal characteristics

Germination, fermentation, and combined germination and fermentation significantly increased onset (64.22–70.52 °C), peak (75.14–81.23 °C) and conclusion temperatures (79.14–85.17 °C) of brown finger millet while endothermic enthalpy (ΔH) decreased (6.85–5.02 J/g). The increase in onset, peak and conclusion temperatures could be associated with the increase in acid concentrations, as observed by reduced pH after the processes (Table 1) which promoted modifications of the starch granules. Further to this is the starch-sugar interactions during the germination and fermentation processes leading to competition for water and modification of the thermal properties. Studies have equally reported such observations, ascribing this to the increase of starch molecular interaction as well as the interplay between starch and other food constituents such as proteins, sugars as well as fats (Chinma et al., 2014; Olamiti, Takalani, Beswa, & Jideani, 2020; Singh & Singh, 2003). Germinated-fermented finger millet flour had the highest gelatinization temperatures followed by fermented, and germinated samples, and vice-versa for ΔH. Thermal results obtained in the present work were in line with increased gelatinization temperatures and decreased ΔH after germination (60 h) of brown rice and adlay flours (Xu et al., 2017) and fermentation (72 h) of koreeb seed flour (Ahmed, Xua, Sulieman, Mahdi, & Na, 2019b). The increase in gelatinization temperatures may be attributed to modification of proteins during germination and/or fermentation that resulted to production of amino acids (Ahmed et al., 2011; Setia et al., 2019). It has been reported that some amino acids increase starch gelatinization temperature (Xu et al., 2017). In addition, changes in starch gelatinization temperatures have been associated with protease and α-amylase activity, amylose, protein and sugar content (Wu et al., 2013; Xu et al., 2017). Endothermic enthalpy, decreased from 6.85 J/g (raw finger millet) to 5.02 J/g (germinated and fermented FM), suggesting that raw finger millet has higher crystallinity and molecular order than the bioprocessed FM flour samples. The ΔH of the processed finger flours are in the order: germinated FM > fermented FM > germinated-fermented FM.

4. Conclusions

Germination and solid-state fermentation have been found as a natural means of improving the functionality and constituents of brown finger millet flour. Germination and solid-state fermentation increased the nutrient composition, amino acid content, total phenolic content, antioxidant activity, and digestibility of brown finger millet flours with low residual antinutrients. Germination and solid-state fermentation increased the water absorption capacity and protein solubility, with changes in pasting and thermal properties. Combined germination and fermentation had the highest influence on brown finger millet which led to enhanced functional properties, nutritional composition and antioxidant activities of the flour. The increased levels of these constituents in the bioprocessed finger millet flours suggest their potentials as functional ingredients in the development of novel bakery products and other food applications. Further studies is recommended for the development of novel gluten-free products prepared from the flours.

CRediT authorship contribution statement

Shakirah Omotoke Azeez: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Chiemela Enyinnaya Chinma:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing. **Stella Oyom Bassey:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Ukamaka Roseline Eze:** Data curation, Formal analysis, Writing – original draft. **Ayodamola Folake Makinde:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Aisha Aderonke Sakariyah:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Sewuese S. Okubanjo:** Methodology, Project administration, Writing – original draft. **Nahemiah Danbaba:** Methodology, Project administration, Writing – original draft. **Oluwafemi Ayodeji Adebo:** Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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