Nutritional and functional attributes of mungbean (Vigna radiata [L] Wilczek) flour as affected by sprouting time

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Abstract
Sprouting of grains improves their nutritional value and functionality, but information on the appropriate sprouting time required to obtain an optimum quality of mungbean flour is limited. This study evaluated the attributes of mungbean flour as influenced by sprouting time. Mungbean seeds were cleaned, sorted, surface-sterilised, rinsed and sprouted (28°C and 26% R.H) for 24 to 120 hr. Proximate, amino acids (AA), vitamins, mineral, anti-nutritional (phytate, tannin, oxalate, trypsin inhibitor, raffinose and stachyose) composition, functional properties (viscosity, bulk density and swelling index), microbial quality (total plate and mould counts) and energy of the flours obtained from the sprouted seeds were analysed. Data were subjected to analysis of variance and the means separated by Duncan’s Multiple Range Test. Significant (P < 0.05) differences were observed in the energy contents, chemical and functional properties of mungbean flour. There was no fungal growth in the samples until after 72 hr. Leucine, followed by lysine, was the dominant essential AA while methionine was the least. In conclusion, increase in sprouting period improved the nutrient composition but reduced the anti-nutrients of mungbean flour. Samples sprouted for 24 hr had the highest total essential and conditionally essential AA.

KEYWORDS
anti-nutrients, functional properties, mungbean, nutritional properties

1 | INTRODUCTION

Legumes rank second after cereals as an essential source of food worldwide and are significant supply of protein in developing countries (Onwurafor et al., 2014). According to Mensah and Olukoya (2007) mungbean (Vigna radiata), popularly called olaludi by the Igbo tribe of Nigeria, is an underutilised legume with high nutritional potentials. It contains appreciable quantity of lysine, and can therefore be used to complement cereals (Onwurafor et al., 2014). The protein, carbohydrate, fat, fibre and ash contents of mungbean are 22.9%, 61.8%, 1.2%, 4.4% and 3.5%, respectively (Offia & Madubuike, 2014). Unlike most other legumes, consumption of mungbean results in little flatulence because of the easy digestibility of the protein and carbohydrate (Nair et al., 2013). Generally, the consumption of mungbean and sprouts maintains the microbial flora in the gut, and reduces the risks of toxic substance absorption, hypercholesterolemia, coronary heart disease and cancer (Ganesan & Xu, 2018). In Eastern Nigeria where it is commonly grown, mungbean can be eaten alone or in combination with yam, cocoyam or abacha.

Sprouting, as a basic pre-processing operation, results in the improvement of edibility, nutritional and functional properties of legumes and cereals (Eleme et al., 2011; Elkhalifa & Bernhardt, 2010;
Ozumba et al., 2002; Zhang et al., 2012). According to Shah et al. (2011) the protein, crude fibre, ash, and vitamin C contents of mungbean increased while the fat, carbohydrate and phytic acid reduced throughout the 96 hr of sprouting. However, Afam et al. (2016) revealed that all the other nutrients (protein, fibre, calcium, iron, magnesium and potassium) except fat, ash, carbohydrate, phosphorus, sodium, flavonoids and antinutrients increased during a 72-hr sprouting of mungbean. Duration is significant in assessing the impact of sprouting on the properties of grains, and enzymatic activities have been reported to reduce when sprouting went beyond 96 hr (Nkhata et al., 2018). According to El-Adawy et al. (2003) and Elkhalifa et al. (2010) sprouting of cereal grains for 120 hr has desirable influence on their functional properties. Furthermore, Helland et al. (2002) who germinated maize grain for 7 days reported increase in germination period led to increased production of α-amylase and reduced viscosity. Little or no information exists on the influence of sprouting on the functional, microbial and amino acid profile of flour obtained from Nigerian mungbean.

This study therefore determined the nutrient and functional attributes of mungbean flour as influenced by sprouting.

## MATERIALS AND METHODS

### 2.1 Procurement and preparation of sample

Mungbean seeds were obtained from a local market in Enugu, Nigeria. The seeds were sorted, sterilised, washed and sprouted in duplicate for 24, 48, 72, 96 and 120 hr. They were dry-milled and sieved with 60 mesh size screen to obtain the flour (Figure 1).

### 2.2 Chemicals and reagents

The chemicals, which were all of analytical grade, and the standards of β-carotene, ascorbic acid, vitamins (B1 and B2) were purchased obtained from Sigma–Aldrich.

### 2.3 Analyses of samples

#### 2.3.1 Proximate

The methods described by AOAC (1990) were used to determine the proximate [crude protein by Kjeldahl method using a Kjeltec (Tecator TM, 91716369) (method 978.04), crude fat by soxhlet extraction (method 930.09), crude fibre by defatting, extraction and ashing (method 930.10), ash incinerating in a muffle furnace (Gallenkamp, SG93/11/888) (method 930.05), moisture by drying at 105°C in an oven (Genlab DC 500, 12B154) (method 930.04)]. The carbohydrate content was determined by subtracting the summation of the values of crude protein, crude fat, crude fibre, ash and moisture from 100 while the energy value was determined by multiplying the values of crude protein, crude fat and carbohydrate by 4, 9 and 4 respectively (Bakare et al., 2020).

#### 2.3.2 β-Carotene

For β-Carotene the modified method of Pearson (1076) was used. About 2 g of mung bean flour was put in a flat bottom reflux flask, followed by the addition of 10 ml of distilled water. The content was shaken with care to form a suspension. About 25 ml of 10% KOH in methanol (v/v) solution was added, and with the mounting of a reflux condenser, the flask and its content were heated in a water bath (70–80°C) for 1 hr. The flask was shaken frequently during the heating. On rapid cooling of the flask, 30 ml of water was added. The flask's content was transferred into a separating funnel and extracted three times with 250 ml of chloroform. About 2 g of anhydrous sodium sulphate was employed to remove traces of water. Thereafter, the flask's content was filtered (using Whatman filter paper No. 42) into a 100-ml volumetric flask, and chloroform was added until the 100 ml was reached. Standard solutions of β-carotene (0–50 μg/ml) were prepared. The absorbance of each of the standard solutions was...
taken, from which a standard graph of absorbance against concentration was constructed, and the slope was calculated as the ratio of the absorbance to concentration. The absorbance of the sample solution was read on a Methrohm Spectronic 21 D Spectrophotometer (Gallenkamp, UK) at 328 nm.

\[
\beta - \text{carotene (µg 100 g}^{-1}) = \frac{\text{absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{weight of sample} \times \text{concentration}}
\]

\[
\text{gradient factor} = \text{slope} = \frac{\text{absorbance}}{\text{concentration}}
\]

2.3.3 | Ascorbic acid, vitamin B1 and vitamin B2

Ascorbic acid was determined by titrimetry as described by Onwuka (2005). A known weight of the sample was mixed with 3% meta phosphoric acid, filtered using a Whatman filter No. 3, and titrated with a standardised solution of 2.6-dichlorophenolindophenol to a faint pink endpoint.

Vitamin B1 was determined according to the modified method of Pearson (1976). About 25 ml of 0.1 M H2SO4 was added to a 100-ml volumetric flask containing 1-g sample. Additional 25 ml of 0.1 M H2SO4 was used to wash down adhering particles on the flask. The flask was put on a boiling water bath to ensure a complete dissolution of the sample in the acid. The flask was shaken frequently in the first 5 min and subsequently every 5 min for 3 hr. The flask was then cooled under running water to less than 50°C. The flask was stoppered and kept at 45–50°C for 2 hr. About 5 ml of taka-diastase in 0.5 M C2H3NaO2 solution was added. Thereafter, the flask and its content were made up to 100 ml with water in the dark after mixing thoroughly. The mixture was filtered (Whatman filter No. 42), and 10 ml of the filtrate was transferred into a 50-ml volumetric flask. Five millilitres of acidic potassium chloride solution (8.5 ml of conc. HCl diluted with 25% [w/v] potassium chloride solution) was then added, shaking thoroughly to mix well. Standard thiamine solutions of range 10–50 mg ml⁻¹ were prepared. The absorbance of both the standard and samples was using at 285 nm using the spectrophotometer.

Vitamin B1 = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{weight of sample}}

For vitamin B2 the method described by Onwuka (2005) was used with modification. About 1 g of sample was put in a 250-ml volumetric flask. Five millilitres each of 5 N HCl and dichloroethene were added sequentially. On shaking the mixture, 90 ml of deionised water was added. The mixture was thoroughly mixed and heated on a water bath for 30 min so as to extract all the riboflavin. This was followed by filtration and cooling. The absorbance of sample and standard was read on a fluorescence Spectrophotometer (DS-11 FX Series, DeNovix Inc) at 460-nm wavelength.

2.3.4 | Minerals

For mineral determination method 975.03 of AOAC (1990) was used. The sample was dried by initially drying in an oven at 70–80°C for 2 hr, and then at 105°C until weight was constant. Wetashing of the sample was done by adding 10-ml nitric-perchloric acid (2:1, v/v) to a flask containing 5-g sample. The flask was heated until a clear digest was obtained. This was followed by cooling and filtration through a Whatman No 1 filter paper. The filtrate was diluted to 100 ml in measuring cylinder. Ca, Mg, Fe and Zn were analysed on atomic absorption spectrophotometer (54 AAS series, GE712354) using air-acetylene gas mixture as oxidant.

Calculation: mg/kg sample = digest conc. X DF (Analyte reading on AAS)

Potassium was analysed using Model 410 Corning Clinical Flame Photometer (2655-00).

Calculation: mg/kg sample = digest conc. X DF (Analyte reading on the photometer)

For the determination of phosphorus, 25 ml distilled water added to a flask containing 5 ml of the digest. Within 5 min, 10 ml of vanadomolybdate reagent was transferred to the flask, followed by mixing. The P content was obtained from the standard curve obtained from plotting absorbance against concentration.

2.3.5 | Plate count

The plate count was done using the method described by Jideani and Jideani (2006) while the fungal and coliform counts were carried out using the method of Harrigan and McCance (1976). For each microbial determination, serial dilutions (10⁻¹ to 10⁻⁴) of the sample were made with Ringers solution. About 1 ml of each dilution was added to a Petri dish containing 15 ml of the appropriate media (nutrient agar for plate count, Sabroud Dextrose agar for fungal count and Mac-Conkey for coliform count). The petri dish was shaken in a circular movement for 10 s. The plates were then allowed to set and incubated (inverted) in incubator (Gallenkamp, SG-94/02/853) for 24 hr at 38°C for plate count, 72 hr at 38°C for fungal count and 48 hr at 38°C for coliform count. The colonies formed were counted and recorded as colony forming unit (cfu) per gram.

Number of colonies = \frac{\text{average count} \times \text{dilution factor}}{	ext{weight of sample}}

2.3.6 | Viscosity, bulk density and swelling power

The methods described by Onwuka (2005) were used to determine the viscosity and bulk density while the swelling power was
determined by the method described by Ikegwu et al. (2010). The viscosity using a viscometer (Brookfield DV-E, RVDVE230) was measured by mixing sample of mung bean flour with water at a ratio of 1:1.

\[
\text{Viscosity at } 30^\circ C (ml/s) = \frac{\text{Volume of flow to maximum time at } 30^\circ C}{\text{maximum time used at } 30^\circ C}
\]

A previously weighed measuring cylinder was filled to the 10 ml mark with the sample. The bottom of the cylinder was tapped gently but repeatedly on a laboratory bench until there was no further reduction of the sample level at the 10-ml mark. The cylinder with the sample was weighed. The bulk density of the samples was determined by using the formula:

\[
\text{BD (g/ml)} = \frac{W_2 - W_1}{V}
\]

where BD = bulk density in g/cm³; W₁ = weight of empty cylinder (g); W₂ = weight of cylinder + sample (g); V = Volume of cylinder occupied by the sample (ml).

One gram of the flour sample was mixed with 10-ml distilled water in a centrifuge tube and heated at 80°C for 30 min under continued shaking. After heating, the suspension was centrifuged (Gallemkamp, 90–1) at 1000 × g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as follows:

\[
\text{Swelling power (g/g)} = \frac{\text{weight of the paste}}{\text{weight of dry flour}}
\]

2.3.7 | Phytic acid, oxalate, trypsin inhibitor activity and tannin

The phytic acid, oxalate, trypsin inhibitor activity, tannin and the oligosaccharides were determined following the methods described by Maga (1983), Onwuka (2005), Kakade et al. (1974), Swain (1979) and Tanaka et al. (1975), respectively. The determination of the phytate involved the extraction of sample with HCl and titration of the extract with acidic solution of FeCl₃ in the presence of ammonium thiocyanate. The oxalate determination involved the digestion of the sample with HCl; oxalate precipitation with conc. NH₄OH, 5% CaCl₂ and phytate. The oxalate determination involved the digestion of the sample with acidic solution of FeCl₃ in the presence of ammonium thiocyanate. The oxalate determination involved the digestion of the sample with HCl; oxalate precipitation with conc. NH₄OH, 5% CaCl₂ and permanganate titration. The oxalate content was given by the relationship that 1 ml of 0.05 m KMnO₄ solution = 0.00225 g oxalate. The oxalate content was calculated using the formula:

\[
\% \text{oxalate} = 100 \times \frac{\text{titre} \times 0.00225 \times W}{2}
\]

where W = Weight of sample used

The determination of trypsin inhibitor involved the centrifugation of the suspension of the sample and phosphate buffer; digestion with 2% casein solution; termination of the digestion with 5% trichloroacetic acid; and measurement of the absorbance at a wavelength of 280 nm on a spectrophotometer. The Folin–Denis spectrophotometric method was used for the determination of tannins. A known weight of sample was measured into a 50-ml beaker of 50% methanol, covered with paraffin and placed in a water bath at 77–80°C for 1 hr. It was shaking thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No. 1 filter paper into a 100-ml volumetric flask, 20-ml water added. 2.5-ml Folin–Denis reagent and 10 ml of 17% Na₂CO₃ were added and mixed properly. The mixture was made up to the 100-ml mark with water, mixed well and allowed to stand for 20 min. The absorbance of the samples was read a Spectronic 21D spectrophotometer at a wavelength of 760 nm.

2.3.8 | Starchose and raffinose

For the determination of starchyose and raffinose, a known weight of the sample was suspended in 80% ethanol, refluxed for 1 hr, filtered through Whatman No. 1 filter paper, and washed further with 80% ethanol. The combined filtrate was evaporated in a rotary vacuum evaporator at 40°C, freeze dried and re-suspended in 10 ml of distilled water. Ten microlitres of the suspension was spotted on triplicate on chromatographic plates (19 × 19 cm) precoated with cellulose powder-G. The plates were kept in a chromatographic chamber containing n-propanol: ethyl acetate: water (6:1:3) as the solvent system. The developed plates were sprayed with 1% α-naphthol in ethyl alcohol containing 10% orthophosphoric acid to locate the sugar spots. The quantitative estimation involved the elution of the spot in distilled water overnight, mixing 1 ml of eluent with 1 ml of 0.02 M thiobarbituric acid and 1 ml of concentrated HCl, heating the mixture in a boiling water bath, and cooling under running water. The resulting yellow colour solution was read at 432.5 nm in a spectrophotometer.

Benitez (1989) method was adopted to determine the amino acid profile using the Applied Biosystems PTH Amino Acid Analyzer. About 500 mg of the sample was put in extraction thimble and extracted for 15 hr in soxhlet extraction apparatus. Nitrogen was determined using the Kjeldahl method (digestion of the sample with H₂SO₄ and Na₂SO₄, distillation, neutralisation with NaOH and titration with HCl). About 30 mg of the defatted sample was weighed into glass ampoules. Thereafter, 7 ml of 6 mol/L HCl was added and oxygen expelled by passing nitrogen gas into the samples. The glass ampoules were sealed with a Bunsen flame and placed in an oven at 105 ± 5°C for 22 hr, and then allowed to cool. The content was filtered and the filtrate evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in a plastic specimen bottle kept in the deep freezer. Then the hydrolysate was dispensed into the cartridge of the analyser.
2.4 | Statistical analysis

Triplicate data were analysed using one-way analysis of variance. Duncan’s Multiple Range Test of the SPSS version computer software 20 was used to separate the means of the data. The significance of the determinations was accepted at \( P < 0.05 \).

3 | RESULTS AND DISCUSSION

3.1 | Effect of sprouting on the proximate composition of mungbean flour

Except for carbohydrate and fat, the proximate composition of mungbean flour increased as the sprouting period increased (Table 1). Table 1 also revealed that as the sprouting period progressed the calorie content of the flour decreased. The enhancement of the protein may be a result of the bio-synthesis of proteases during the sprouting process (Afam et al., 2016; Kaushik et al., 2010) and the hydrolysis of the protein-enzyme-mineral bond resulting in the release of the nutrients (Elemo et al., 2011; Nonogaki et al., 2010; Shah et al., 2011). This result is in line with the findings of many other authors (Afam et al., 2016; Camacho et al., 1992; Ghavidel & Prakash, 2007; Kaushik et al., 2010; Ohtsubo et al., 2005; Urbano et al., 2005; Shah et al., 2011). The fact that fat is utilised as a source of energy may due to its decrease during sprouting (Afam et al., 2016; Devi et al., 2015; El-Adawy, 2002; Ghavidel & Prakash, 2007; Onimawo & Asugo, 2004; Shah et al., 2011). Fibre is an essential part of the diet that regulates bowel movement and weight. The increase in the crude fibre and ash contents of the flour is supported by Shah et al. (2011) and Devi et al. (2015), respectively. Moisture content increased slightly as sprouting increased. The increase in the moisture content with time may be due to an increase in the number of cells within the seed becoming hydrated (Nonogaki et al., 2010). However, the rate at which seeds imbibe water during sprouting varies with time (Devi et al., 2015), thus the varying moisture content. Uwaegbute et al. (2000), Shah et al. (2011) and Devi et al. (2015) also reported moisture increases during the sprouting of legumes. However, a contradictory result was observed by Ohtsubo et al. (2005) during the sprouting of brown rice. The reduction of the carbohydrate contents of the flour samples is in line with the findings of Jirapa et al. (2001), Inyang and Zakari (2008), Megat Rusydi et al. (2011) and Uppal and Bains (2012). The decrease in carbohydrate during sprouting might be due to the catabolic action of \( \alpha \)-amylase (Shah et al., 2011). According to Vidal-Valverde et al. (2002) carbohydrate is used as source of energy for the growing seedlings during sprouting. The observed decrease in carbohydrate promotes sprouted mungbean seeds as a useful diet in weight management or any other diet-related health condition requiring low carbohydrate consumption. The decline in the energy level of the flour samples as sprouting time increased, which was also reported by Kalimbira et al. (2004), may be due to its use for the metabolic activities of the young shoot. This low energy makes mungbean sprouts beneficial for individuals with obesity and diabetes (Zheng, 1999).

3.2 | Sprouting effect on the vitamin content of mungbean

Sprouting leads to the activation of several enzyme systems which brings about notable changes in the chemical constituents of legumes. As presented in Table 2, there were significant \( P < 0.05 \) increases in

| Sprouting time (hr) | \( \beta \)-carotene (\( \mu \)g/100 g) | Vitamin C (mg/100 g) | Vitamin B1 (mg/100) | Vitamin B2 (mg/100 g) |
|---------------------|-----------------|-----------------|-----------------|-----------------|
| 0                   | 115.72          | 2.65            | 0.21            | 0.03            |
| 24                  | 119.18          | 2.87            | 0.27            | 0.05            |
| 48                  | 121.13          | 2.95            | 0.35            | 0.03            |
| 72                  | 123.71          | 3.12            | 0.41            | 0.06            |
| 96                  | 125.47          | 3.19            | 0.43            | 0.06            |
| 120                 | 127.06          | 3.22            | 0.47            | 0.07            |

Note: Means in a column having same alphabets are not significantly different at \( P > 0.05 \).
the β-carotene, vitamins C, B1 and B2 contents of the sample as the sprouting period increased, and this agrees with previous reports (Fernandez & Berry, 1988; Riddoch et al., 1998; Shah et al., 2011; Uppal & Bains, 2012; Vidal-Valverde et al., 2002; Yang et al., 2001). Germination has been reported to result in the synthesis of water soluble vitamins (Bibi et al., 2008; Nkhata et al., 2018).

3.3 | Effect of sprouting on the mineral content of mungbean flour

Table 3 shows that there were significant increases in the mineral content (Ca, Zn, P, Mg, Fe and K) of the flour samples. Previous researchers (Afam et al., 2016; Dave et al., 2008; Devi et al., 2015; Tizazu et al., 2011) also reported increases in mineral contents of legumes during sprouting, and this may be due to increase in the activity of phytase, which breaks down protein-enzyme-mineral bond to release the minerals (Abdelrahaman et al., 2007; Elemo et al., 2011). Nout and Motarjemi (1997) attributed the increase in mineral contents to the decrease in the antinutrients during sprouting. On the other hand, sprouting had been reported to have no significant influence on the iron content of cowpea (Bains et al., 2011; Devi et al., 2015).

| Sprouting time (hr) | Ca (mg/kg) | Mg (mg/kg) | Fe (mg/kg) | Zn (mg/kg) | P (mg/kg) | K (%) |
|---------------------|------------|------------|------------|------------|-----------|-------|
| 0                   | 1705.48a   | 311.14a    | 460.49a    | 21.32a     | 527.43a   | 1.17a |
| 24                  | 1995.87b   | 313.43a    | 477.21b    | 22.27b     | 666.62b   | 1.30b |
| 48                  | 2263.67c   | 316.81b    | 481.63b    | 23.83c     | 701.73c   | 1.39c |
| 72                  | 2483.94d   | 319.10b    | 627.88c    | 23.83c     | 775.45d   | 1.56d |
| 96                  | 3599.60e   | 323.87c    | 630.09c    | 25.22d     | 1243.66e  | 1.66e |
| 120                 | 3860.92f   | 328.55d    | 1163.10d   | 27.59e     | 2918.06f  | 1.75f |

Note: Means in a column having same alphabets are not significantly different at P > 0.05.

3.4 | Effect of sprouting on the antinutrient composition of mungbean flour

Table 4 shows that the phytate, oxalate, trypsin inhibitor, raffinose and starchyose decreased as the sprouting time increased. Tannin was not detected in the flour samples. The enzymatic break down of phytate-phosphorus during sprouting may have resulted in the decrease in the phytic acid (Gupta et al., 2015; Shah et al., 2011). According to Murugkar et al. (2013), oxalic acid is broken down to carbon (IV) oxide and hydrogen peroxide during sprouting and subsequent release of calcium. The reduction in the trypsin inhibitor may be due to the proteolytic activity of enzymes during sprouting (Chauhan & Chauhan, 2007). Several other workers (Chopra & Sankhalla, 2004; Devi et al., 2015; Modgil et al., 2009; Uppal & Bains, 2012) also reported that antinutrients of legumes decreased as sprouting period increased. Murugkar and Jha (2009) similarly reported a decrease in trypsin inhibitor activity during sprouting of soybean. The non-detection of tannin in the samples might be a result of the development of hydrophobic association of tannins with proteins and enzymes and the subsequent leakage of tannin into water (Afam et al., 2016; Megat Rusydi & Azrina, 2012). Adeleke et al. (2017) similarly reported a reduction in the tannin content of sprouted Bambara nut. The decrease in the raffinose and starchyose of the flour samples may be due to the enzymatic attack of starchyose and raffinose by galactosidase during sprouting (Adeleke et al., 2017). This observation is in line with the findings of Nnanna and Phillips (1988) and Jood et al. (1985). Adeleke et al. (2017) reported considerable losses of stachyose and raffinose in Bambara groundnut flour after 72 hr of sprouting which resulted in better digestibility.

| Sprouting time (hr) | Phytate (%) | Oxalate (%) | Trypsin inhibitor (TUI/mg) | Tannin (%) | Raffinose (%) | Starchyose (%) |
|---------------------|-------------|-------------|----------------------------|------------|---------------|---------------|
| 0                   | 0.18f       | 0.13c       | 31.77f                     | 0.01       | 1.50a         | 1.92a         |
| 24                  | 0.15e       | 0.12bc      | 28.92e                     | ND         | 1.41d         | 1.78c         |
| 48                  | 0.13d       | 0.11b       | 27.88d                     | ND         | 1.34c         | 1.76c         |
| 72                  | 0.13c       | 0.11b       | 26.29c                     | ND         | 1.29b         | 1.65b         |
| 96                  | 0.12b       | 0.10a       | 22.20b                     | ND         | 1.25ab        | 1.59b         |
| 120                 | 0.10a       | 0.09a       | 19.87a                     | ND         | 1.21a         | 1.49a         |

Note: Means in a column having same alphabets are not significantly different at P > 0.05.
degradation by α- and β-amylases that are formed during germination (Helland et al., 2002). The bulk density, which is a measure of heaviness of flour as well as a determinant of the packaging requirement of food (Adebowale et al., 2005; Murugkar et al., 2013; Nicole et al., 2010) decreased as the sprouting time increased. This agrees with the reports of Elkhalifa and Bernhardt (2010) and Ocheme et al. (2015). This reduction may be attributed to the fact that starch and proteins are broken down during sprouting (Ocheme et al., 2015). There was a decrease in the swelling power of the flour samples as the sprouting time increased. Similar decrease was reported by Gernah et al. (2011) during the sprouting of maize, and this may be due to the dextrinization of the starches.

### 3.6 Effect of sprouting on the microbial count of mungbean flour

The total plate and fungal counts of the samples increased with increase in sprouting time (Table 6). Yang et al. (2001) and Dziki et al. (2015) also observed a similar occurrence. Aydin et al. (2009) reported that although flour has a low water activity, the indigenous microbial population is diverse and their activities are triggered by the warm and humid conditions characteristic of sprouting.

### 3.7 Influence of sprouting on the amino acid composition of mungbean flour

Protein quality is influenced by amino acid pattern (Ayalew et al., 2017). There was an increase in the total essential amino acids (TEAA; valine, tryptophan, histidine, isoleucine, leucine, methionine, phenylalanine, lysine and threonine) as sprouting time increased (Table 7). This agrees with Mubarak (2005). This increase may be due to the breakdown of protease-resistant prolamin protein, releasing some amount of amino acids (Afify et al., 2012). Leucine in the range from 6.48 to 8.33 g/100 g was the dominant essential amino acid while methionine (0.85 to 1.85 g/100 g) was the least essential amino acid in all the samples. Among the conditionally essential amino acid, arginine (5.33 to 7.74 g/100 g) was the most abundant amino acid while cystine (0.61 to 1.45 g/100 g) was the least concentration in all the samples. Regarding the non-essential amino acids, glutamic acid (10.75 to 17.50 g/100 g) was dominant while alanine (3.41 to 4.48 g/100 g) was the least amino acid.

The TEAA ranged from 30.13 to 35.67 g/100 g with the flour sample obtained from 24-hr sprouted seed having the highest. Mubarak (2005) also reported 38.6 g/100 g as the TEAA for mung bean seeds germinated for 72 hr. On the other hand, Grela et al. (2017) reported 33.61 to 40.55 g/100 g for some legume species (chickpea, broad bean, grasspea and pea). However, Ayalew et al. (2017) reported that the TEAA of Anchote tuber and leaf powder ranged from 17.10 to 29.28 g/100 g. The total conditionally essential amino acids ranged from 16.85 to 23.67 g/100 g with 96-hr sprouted sample having the least. The total non-essential amino acids ranged from 27.79 to 40.21 g/100 g with the control having the least. Sprouting at 24 hr showed the highest concentration of both essential amino acids and conditionally essential amino acids, while sprouting at 72 hr showed the highest non-essential amino acids. The total amino acid content of the samples ranged 75.02 to 95.31 g/100 g with the 24-hr sprouting time having the highest concentration and control having the least concentration.
The results of the conditionally essential and non-essential amino acid concentration in this study compares favourably with most vegetable proteins (Mune et al., 2011).

4 | CONCLUSIONS

Increase in sprouting period resulted in increase in protein, crude fibre, ash, moisture, vitamins A, C, B1, B2, calcium, magnesium, iron, zinc, phosphorus, and potassium, as well as the microbial count, of mungbean flour. On the other hand, increase in sprouting period led to decrease in crude fat, carbohydrate, energy, phytate, oxalate, trypsin inhibitor, tannin, raffinose and starchyose contents of mungbean flour. The functional properties (viscosity, bulk density and swelling power) of mungbean reduced as sprouting period increased. Highest microbial count (total plate count and mould count) was observed in the flour sample obtained from mungbean sprouted for 120 hr. Mungbean flour sprouted for 24 hr had the highest TEAA and total conditionally essential amino acid, although flour sample sprouted for at least 96 hr was richer in protein, while 120-hr sprouted sample had the highest contents of fibre, ash, ascorbic acid and minerals elements.

CONFLICT OF INTEREST
All the authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS
Chinelo Elobuike: investigation, methodology. Michael Idowu: conceptualization, methodology, project administration, supervision, validation, writing-original draft, writing-review & editing. Abiodun Adeola: conceptualization, formal analysis, investigation, methodology, project administration, supervision, writing-original draft, writing-review & editing. Henry Bakare: conceptualization, formal analysis, investigation, methodology, project administration, supervision, writing-review & editing.

DATA AVAILABILITY STATEMENT
The corresponding author declares the availability of data upon reasonable request.
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