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Effect of sprouting on the nutrient and functional properties of *Sorghum bicolor* and *Vigna subterranean* flour blends for weaning formula

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ABSTRACT

This study focused on the effect of sprouting on the nutrient and functional properties of sorghum and Bambara nut flour blends for weaning formula. The sorghum and Bambara nut were sprouted separately for seven days and the sprouts were measured for nutrients, anti-nutrients and amino acid levels. Sprouting for 4 and 5 days optimized the nutrient contents of Bambara nut and sorghum respectively. The seeds of Bambara nut contained a higher concentration of all the amino acids except for isoleucine (3.60 g/100 g protein) and threonine (2.05 g/100 g protein). Sprouting significantly reduced the levels of oxalate and phytate by 38.78 % and 22.38 % respectively in Bambara nut and by 39.44 % and 19.23 % in Sorghum. Gelatinization temperature, bulk density and foam capacity were significantly lower in sprouted Bambara nut flour however, in sprouted sorghum flour, only gelatinization temperature and time differed significantly ($p < 0.05$) one from another. The formulation of 80 % Sorghum and 20 % Bambara nut flour had significantly lower gelatinization temperature (56.75 ± 0.10 °C), swelling capacity (3.36 ± 0.08 %) and higher gelatinization time (13.76 ± 0.21 s). Formulation of 20 % Bambara inclusion enhanced the protein levels of the blend as such can be used as a supportive diet for growing children.

1. Introduction

The need for nutrient is of more prominence biologically at the early stage of life (childhood) than in adulthood. In adults, the nutrient supply must be available for maintenance and in meeting the body's requirements for physical action. However, in infants and growing children, energy and nutrient requirement is for growth and development. The amount and nature of nutrient supply during early life regulates the separation of tissues and organs and has an immediate and long-term effect on the wellbeing of an individual (Koletzko et al. 2015).

According to WHO (2018) few children receive nutritionally adequate and safe complementary foods; in many countries less than a fourth of infants 6–23 months of age meet the criteria of dietary diversity and feeding frequency that are appropriate for their age. After 6 months of breast feeding, the capability of breast milk to meet supplies for macronutrients and micronutrients (energy, protein, iron, zinc, and some fat-soluble vitamins) gradually turn out to be low especially as the age of the infant increases (Isabelle & Chan, 2011). There are some nutritional gaps that need to be covered by the complementary food being administered to the growing infants. A good complementary food should offer adequate energy, protein and micronutrients to fill up nutritional gaps and meet the infant's needs in addition to breast milk.

Aside the nutritional requirement of weaning foods, the nature as touching texture, taste, colour among others are to be considered also. World Health Organization (2009) recommends that weaning food should be concentrated in energy, nutritious, soft and easy to swallow.

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The family's food on the contrary is often filling and bulky thereby making it difficult for weaning children to digest and hence absorption of nutrient is impaired. In an attempt to address this bottleneck seeds used in formulation of baby foods are being sprouted to improve functional properties of children meals. Ocheme et al. (2015) reported that sprouting of sorghum for 24, 48 and 72 h improved the functional properties and degree of starch gelatinization of the flour using non-germinated sorghum as control. The present study therefore attempts to evaluate the effect of sprouting on the nutrient and functional properties of blends of sprouted *Sorghum bicolor* and *Vigna subterranean* flour as weaning formula.

2. Materials and methods

2.1. Reagents and chemicals

Analytical graded reagents as well as other chemicals which are BDH and Sigma Aldrich products were used in this study except otherwise stated.

2.2. Sprouting/germination

Exactly 20 g of soaked Bambara nut was weighed in 8 different Petri dishes each for different germination periods (0, 1, 2, 3, 4, 5, 6 and 8 days). The same treatment was carried out for sorghum. The samples were covered with paper to create an enabling sprouting environment for the samples. The sprouted Bambara nut was oven dried to a constant weight at 60 °C for 12 h while sorghum was dried to constant weight at 40 °C for 8 h respectively. The dried sample were milled and sieved with a 2 mm mesh size sieve.

2.3. Sample preparation

Sorghum and Bambara nut obtained from the market were thoroughly cleaned by separating the seeds from stones and other unwanted materials. Sorghum and Bambara nut were separately washed and soaked in clean water for a period of 12 and 15 h respectively. At an interval of 3 h, the water was constantly changed to avoid the seeds from becoming fermented. After the soaking time, the water was drained and the samples allowed to sprout after being spread on trays (Modu et al., 2014; James et al., 2018).

2.4. Proximate analyses

The proximate composition of the flours (sprouted Bambara nut and sorghum) and their corresponding supplemented diets were carried out in triplicate. Methods used are described below.

2.4.1. Determination of moisture content

The method described by Onwuka (2005) was used to determine the moisture content. Moisture content is determined through a thermo-gravimetric method that is by loss on drying, a principle in which the sample is heated and the weight loss due to evaporation of moisture is recorded. This was carried out by oven drying method. Two grams (2 g) of well-mixed samples was accurately weighed in clean, dried crucible (W_1). The crucible was allowed in an oven at 100–105 °C for 6–12 h until a constant weight was obtained. The crucible was then cooled for 30 min. After cooling it was weighed again (W_2), the percentage moisture was calculated by following formula.

$$Moisture\ Content(\%) = \frac{W1 - W2}{Weight\ of\ the\ Sample} \times 100 \quad (1)$$

Where W_1 = Initial weight of crucible + Sample and W_2 = Final weight of crucible + Sample

2.4.2. Determination of fat content

Crude fat was determined by the method described by Onwuka (2005). The principle is based on based on solvent extraction. Fat is extracted based on the fact that it is soluble in diethyl ether and petroleum ether and hence can precipitate into the solvent. This method involves extraction of liquid (ether) using Soxhlet apparatus. Exactly 2 g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. A weighed, cleaned and dried receiving flask was filled with petroleum ether and fitted into the apparatus. The Soxhlet apparatus was assembled and allowed refluxing for 6 h; extract was transferred into clean glass dish with washing which was evaporated on water bath. Then the dish was placed in an oven at 105–110 °C for 1 h and cooled in a desiccator. The percentage crude fat was determined using the following formula:

$$Crude\ Fat\ Content(\%) = \frac{Weight\ of\ Extract}{Weight\ of\ the\ Sample} \times 100 \quad (2)$$

2.4.3. Determination of carbohydrate content

The nitrogen free method described by AOAC (1990) was used. Carbohydrate content can be measured by hydrolyzing polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharide. The carbohydrate was calculated as weight by difference between 100 and summation of other proximate parameter as Nitrogen free extract (NFE) percentage carbohydrate (NFE) = 100-(M+P + F+A+F_2) where M = moisture, P = protein, F_1=Fat, A=ash, F_2=crude fibre.

2.4.4. Determination of crude protein

Protein in the sample was determined by Kjeldahl method. The principle is based on digestion of organic matter with sulfuric acid in the presence of a catalyst, rendering the reaction product alkaline the distillation and titration of the liberated ammonia, calculation of the nitrogen content, multiplication of the result by the conventional factor 6.25 to obtain the crude protein content.

Exactly 0.25 g of dried flour samples were taken in digestion flask, with 6 mL of concentrated H_2SO_4 and a speck of Kjeldah1 catalyst (mixture of 10 g Na_2SO_4+5 g $CuSO_4$+ 0.05 g selenium). The flask was swirled in order to mix the contents thoroughly then digested on the digestion block till the mixtures became clear (colourless or greenish in color). The digest was cooled and transferred to 100 mL volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markham Distillation Apparatus. Ten millilitres of digest was introduced in the distillation tube then 10 mL of 40 % NaOH was gradually added through the same way. Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in conical flask containing 5 mL of 4 % boric acid solution with few drops of methyl red indicator. During distillation yellowish colour appeared due to the NH_4OH. The distillate was then titrated against standard 0.1 N HCI solutions till the appearance of pink colour. A blank was also run through all steps as above. Percentage crude protein content (% Crude Protein) = 6.25* x %N

$$Protein\ Content(\%) = \frac{(S - B) \times N \quad \times 0.014 \quad \times D}{Weight\ of\ the\ Sample \times V} \times 100 \quad (3)$$

Where S and B = Sample and blank titration values, N = HCl Normality, D = Dilution of sample after digestion, V = Volume taken for distillation, 0.014 – Milli equivalent weight of Nitrogen and * = Correction factor.

2.4.5. Determination crude fibre

The method described by Onwuka (2005) was used in the determination of the crude fibre. Crude fiber is determined gravimetrically after chemical digestion and solubilization of test sample. The principle is based on acid/alkali treatment, oxidative hydrolytic degradation of cellulose and lignin and filtration. The residue obtained after final

filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fiber content. Two grams (2 g) of sample was defatted with petroleum ether; boiled under reflux for 30 min with 200 mL of a solution containing 1.25 g of H_2SO_4 per 100 mL of solution. The solution was filtered through several layers of cheese cloth on fluted funnel, washed with boiling water until the washings are no longer acidic then the residue was transferred into a beaker and boiled for 30 min with 200 mL of solution containing 1.25 g of carbonate free NaOH per 100 mL, the final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, then dried in an electric oven at 105 °C and weighed after which it was incinerated at 550 °C for 30 mins, it was then cooled and reweighed. The loss in weight after incineration x 100 is the percentage crude fibre.

2.4.6. Determination of ash content

To ash any substance, it is based on burning off the organic matter and to determine the inorganic matter remaining. It requires two stages of heating; the first involves removal of the water present and to char the sample thoroughly; and finally, ashing at 550 °C in a muffle furnace, the weight of ash thus obtained is expressed in terms of percentage. The method of Onwuka (2005) was used for the determination of the ash content. Clean empty crucible was placed in a muffle furnace at 550 °C for an hour, cooled in desiccator and then weight of empty crucible was noted (W_1). Two grams of each of the samples was taken in crucible (W_2) and was charred over a burner, until it was charred. Then the crucible was placed in muffle furnace for ashing at 550 °C for 2–4 h. The appearance for gray white ash which indicated complete oxidation of all organic matter in the sample. After ashing the crucible was cooled and weighed (W_3). Percentage ash was calculated by the following formula.

$$Ash\ Content(\%) = \frac{Difference\ in\ Weight\ of\ Ash}{Weight\ of\ the\ Sample} \times 100 \quad (4)$$

Difference in weight of ash = $W_3 - W_1$

2.5. Amino acid profile

The Amino Acid profile in the known sample was determined using methods described by Benitez (1989). The known sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

2.5.1. Defatting of sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. Exactly 500 mg of the sample was put in extraction thimble and extracted for 15 h in Soxhlet extraction apparatus.

2.5.2. Hydrolysis of the sample for tryptophan determination

Two grams (2 g) of the defatted Bambara nut sample was weighed into glass ampoule. Exactly 7 mL of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis example methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 °C ± 5 °C for 22 h. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was neutralized to pH 7.00 and evaporated to dryness at 40 °C under vacuum in a rotary evaporator. The residue was dissolved with 5 mL of borate buffer (pH 9.0) and stored in plastic specimen bottle for further analysis.

2.5.3. Nitrogen determination

Exactly 115 mg of ground sample was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 mL) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added. The flask was then put in Kjeldhal digestion apparatus for 3 h until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 mL in standard volumetric flask. Aliquot (10 mL) of the diluted solution with 10 mL of 45 % sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 mL of 2 % boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 mL of distillate was collected. The distillate was then titrated with standardize (0.01 N) hydrochloric acid until a grey-coloured end point was obtained.

$$Ash\ Content(\%) = \frac{(a-b) \times 0.01 \times 14 \quad \times V}{W \quad \times C} \times 100 \quad (5)$$

Where: a = Titre value of the digested sample, b = Titre value of blank sample, v = Volume after dilution (100 mL), W =Weight of dried sample (mg), C = Aliquot of the sample used (10 mL) and 14 = Nitrogen constant in mg.

2.5.4. Sample loading

Five microliters of sample were dispensed into the cartridge of the analyser. The TSM analyser is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysate. The period of the analysis lasted for 76 min.

2.5.5. Method of calculating amino acid values from chromatogram peaks

The system is an automated one in which an integrator is attached to the Analyzer which calculates the peak area proportional to the concentration of each of the amino acids.

2.6. Functional properties

The functional properties including the bulk density, swelling capacity and solubility index, water absorption capacity, foaming capacity and gelation capacity of the Bambara nut and sorghum flours were determined as follows:

2.6.1. Determination of bulk density

The method reported by James et al. (2018) was used to determine the bulk density. A 10-mL graduated measuring cylinder was weighed and filled with the Bambara nut and sorghum flours, and the bottom of the cylinder was gently tapped on the laboratory bench several times until there was no further decrease of the sample level after filling to the 10 mL mark. This was calculated using the formula;

$$Bulk\ Density = \frac{Weight\ of\ Sample(g)}{Volume\ of\ Sample(mL)} \quad (6)$$

2.6.2. Swelling capacity and solubility index

The method described by AOAC (2000) was adopted in the determination of swelling capacity and solubility index. One gram of the sample was accurately weighed and transferred into a clean dried test tube and weighed (W_1), and then it was dispersed into 30 cm^3 distilled water using blender. The resultant slurry was heated at temperatures of 60, 70, 80, and 90 °C, respectively, for 30 min in a regulated water bath. The mixture was then cooled to room temperature and centrifuged at 500 rpm for 15 min. 5 mL of the supernatant was withdrawn and the residue was the amount solubilized in water.

$$Solubility = \frac{Weight\ after\ Drying\ of\ Supernatant}{Weight\ of\ Sample\ after\ Drying} \quad \times 100 \quad (7)$$

2.6.3. Water absorption capacity

Water absorption capacity was determined using the method of Sathe et al. (1981) with slight modifications. 10 mL of distilled water was added to 1.0 g of the sample in a beaker. The suspension was stirred using a magnetic stirrer for 5 min. The suspension obtained was thereafter centrifuged at 3555 rpm for 30 min and the supernatant measured

in a 10 mL graduated cylinder. The density of water was taken as 1.0 g/cm 3. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant.

2.6.4. Foaming capacity

The foam capacity and stability were studied by the method of Coffman and Garcia (1977). A known weight of sorghum and Bambara nut flour each sample was dispersed in 100 mL distilled water. The resulting solution was homogenized for 5 min at high speed. The volume of foam separated was noted. The total volume remaining at interval of 0.00, 0.30, 1, 2, 3, 4 up to 24 h was noted for the study of foaming stability.

$$Foaming\ Capacity(\%) = \frac{Volume\ after - Volume\ before\ Homonisation}{Volume\ before\ Homogenization} \times 100 \quad (8)$$

The effect of pH on foaming properties was carried out by adjusting 2 % (w/v) dispersion to the desired pH range from 2 to 11 using either 1 M HCl or NaOH followed by vigorous whipping as described above.

2.6.5. Determination of gelation capacity

Method described by Onwuka (2005) was adopted in the determination of gelation capacity. Bambara nut and sorghum flour sample each of 5 % (w/v) in 5 mL of distilled water was prepared in test tubes. The samples in the test tubes were then heated for 1 h in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes were further cooled for 2 h at 4 °C. The least gelation concentration determined is the concentration when the sample from the inverted test tube did not fall or slip.

2.7. Anti-nutritional factors

Tannin, phytates, oxalate, cyanide, saponin and alkaloid contents of sprouted Bambara nut and sorghum were determined using spectrophotometric methods described by Armtfiled et al. (1985)).

2.7.1. Determination of phytates

Phytate content of the samples was determined using the procedure of Lolas and Markakis (1975) as described by Essien and Akpan (2014). Two grams (2 g) of sample was weighed into a 250 mL conical flask. Exactly 100 mL of 2 % concentrated HCl was used to soak the sample in the conical flask for 3 h. The mixture was filtered and 50 mL of filtrate was placed in a 250 mL beaker and 107 mL of distilled water was added. A 0.3 % ammonium thiocyanate (10 mL) was added to the sample as indicator and titrated with iron III chloride solution which contained 1.95 mg iron per mL. Titration continued until a brownish yellow colour that persisted for 5 min was observed.

2.7.2. Determination of tannins

The Folin Denis Spectrophotometric method was employed as described by Onwuka (2005) to determine the tannin. One gram (1 g) of each sample was dispersed in 10 mL distilled water and shaken. The mixture was allowed to stand for 30 min at room temperature. At the end of 30 min, the mixture was centrifuged and the extract obtained. (2.5 mL) of the supernatant (extract) was transferred into a 50 mL volumetric flask. Similarly, 2.5 mL of standard tannic acid solution was transferred into a separate 50 mL flask. One millitre (1 mL) of Folin – Denis reagent was measured into each flask, followed by 2.5 mL of saturated Na_2CO_3 solution. The mixture was diluted to mark in the flask (50 mL) and incubated for 90 min at room temperature. The absorbance was measured at 250 nm using Jenway model 6000 Electronic Spectrophotometer. The tannin content was calculated from:

$$\%\ Tannin = An/As \times C \times 100/W \times Vf \quad (9)$$

Where An = absorbance of test sample, As = absorbance of standard solution. C= concentration of standard solution, W = weight of sample, Vf = total volume of extract.

2.7.3. Determination of oxalates

The titration method described by Day and Underwood (1986) was employed in the determination of oxalate content of the sample. One gram (1 g) of each sample was weighed into a 100 mL volumetric flask, where 75 mL of 3 N H_2SO_4 was added and stirred for 1 h. The mixture was then filtered with Whatman No 1 filter paper. From the filtrate, 25 mL was taken and titrated against 0.1 N KMnO4 solution, until a pink colour persisted for at least 30 s. The oxalate content was calculated as follows:

$$Oxalate(mg \Big/ 100g) = \frac{T \quad \times Vme(DF) \times 105}{Me \times Mf} \quad (10)$$

Where: T = Titre of $KmNO_4$ (mL), Vme is the volume mass equivalent (1 cm^3 of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), Df is the dilution factor (Vt/A =75/25 =3) where Vt is the total volume of filtrate (75 mL) and A is the aliquot used for titration (25 mL) and Me is the molar equivalent of $KMnO_4$ in oxalate and Weight of sample.

3. Results

3.1. Proximate composition of sprouted and unsprouted sorghum and Bambara nut flours

The proximate composition of sprouted and un-sprouted sorghum flour is presented in Fig. 1 (Table S1). The unsprouted sorghum flour had significantly ($p < 0.05$) lower ash (although comparable to day one sprouted sorghum), fat, moisture and protein contents. These parameters increased with increase in sprouting time (day five) until day six where decrease was observed except in the moisture contents of the sprouted sorghum flour which increased at day seven of sprouting. However, the reverse trend was observed for fiber and carbohydrate contents of sprouted sorghum flour. Both parameters decreased with increasing spouting time from day one to five after which increase ensued (at days 6 and seven). At day five of sprouting, sorghum flour had the highest ash (1.10 ± 0.70 %), fat (3.27 ± 0.08 %), moisture (13.40 ± 0.07 %) and protein (10.99 ± 0.01%) contents. The highest fiber and carbohydrate contents were observed at day seven (10.08 ± 0.012 %) and zero (that is unsprouted sorghum) (69.82 ± 0.20 %) respectively.

The proximate composition of Bambara nut sprouted at various duration is presented in Fig. 1 (ST-2). At day 0, Bambara nut seed was not sprouted while days 1–7 were the duration of the sprouting. Ash, fat, moisture and protein contents increased with increase in sprouting time until the 4th day where the highest values (4.13 ± 0.075, 4.22 ± 0.064, 25.67 ± 0.403 and 5.73 ± 0.042 % respectively). Decrease in the afore mentioned parameters was observed from day five through the end of sprouting (seventh day).

Meanwhile, the carbohydrate content in Bambara nut was significantly (62.33 ± 0.587 %) higher at days 0 (unsprouted) with significant decrease through the 4th sprouting day before a subsequent increase from day five till the seventh day ensued. The highest fiber content (9.04 ± 0.073 %) was observed at day seven

3.2. Amino acid composition of Sorghum and Bambara nut

The result presented in Fig. 2 shows that *S. bicolor* and *V. subterranean* seeds contain both essential and non-essential amino acids. The seeds of *V. subterranean* contained a higher concentration of most of the amino acids except for isoleucine and threonine that were higher in the *S. bicolor*. The amino acid (glutamate acid) was most abundant in both seeds. Methionine had the lowest concentration of amino acid in

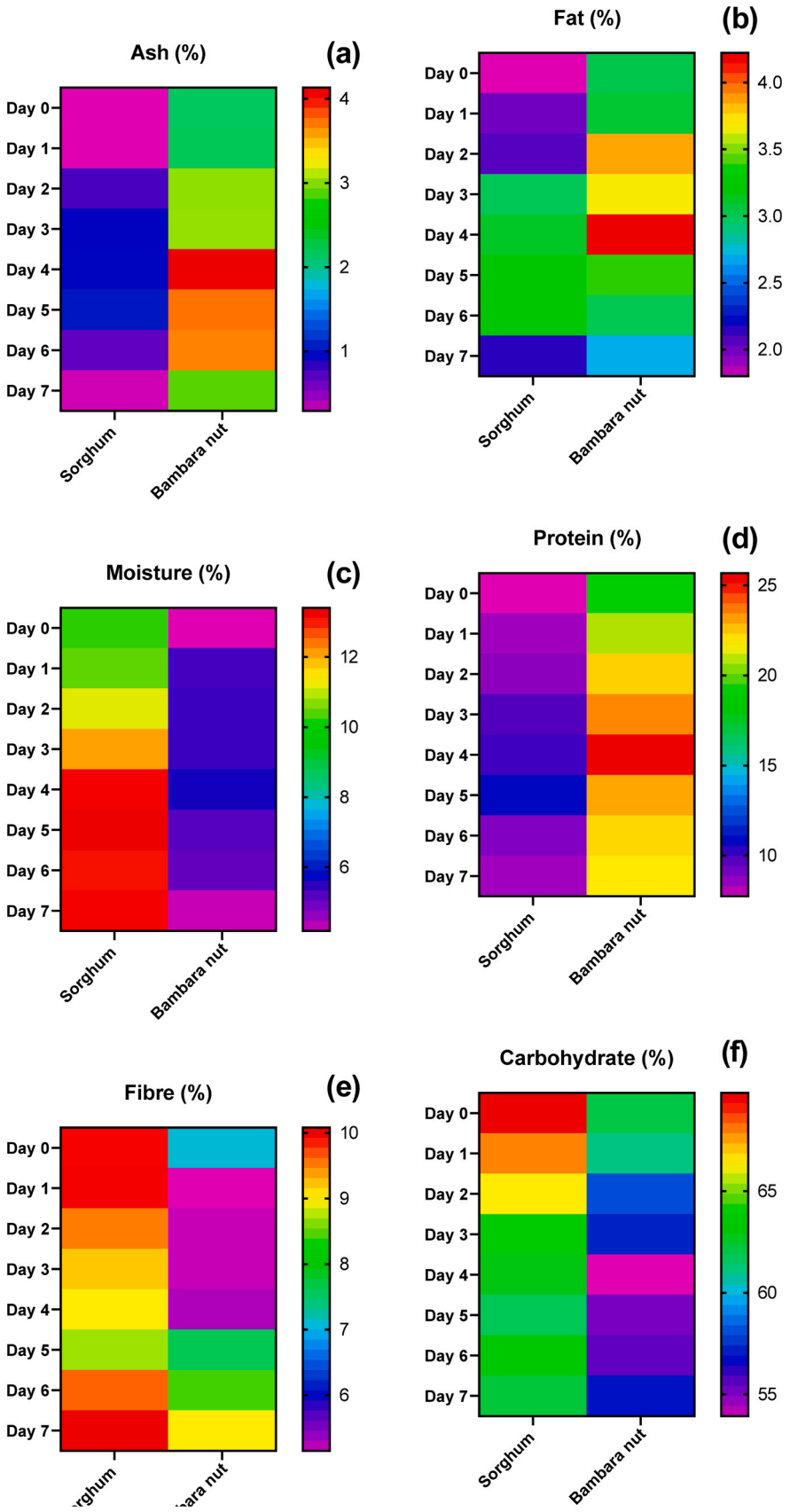

Fig. 1. Proximate Composition (%) of ash (a), fat (b), moisture (c), protein (d), fibre (e) and carbohydrate (f) in Sorghum Flour Sprouted and Bambara Nut Flour sprouted at Varying Durations.

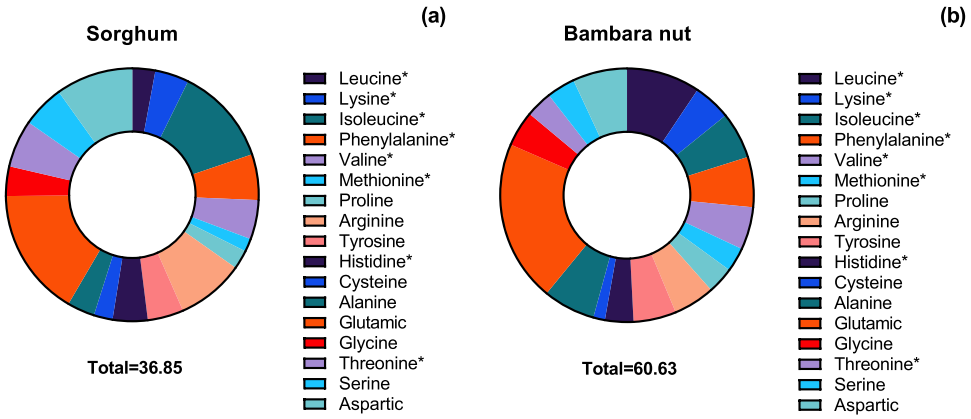

Fig. 2. Amino Acid Composition (g/100 g) of Sorghum and Bambara nut. *Essential amino acid.

sorghum while in Bambara cysteine had the lowest concentration of amino acid.

The result in Fig. 3 (ST-3) shows the different classifications of the amino acid present in sorghum and Bambara nut flours. All the classes of amino acids were significantly ($p < 0.05$) higher in Bambara nut flour compared to Sorghum flour except that the ratio of Total aromatic amino acid (TArAA) and Total non-aromatic amino acid (TNArA) was significantly higher in Sorghum as well as the ratio of Total essential amino acid (TEAA) and Total non-essential amino (TNEAA) being statistically ($p > 0.05$) similar to that of Bambara nut.

3.3. Anti-nutrient composition of sprouted and unsprouted sorghum and bambara nut flours

Presented in Fig. 4a (ST-4) is the influence of sprouting on the anti-nutritional contents of Sorghum. It was observed that increase in sprouting time resulted to significant ($p < 0.05$) decrease from days 0–5 that is from 28.33 ± 0.33 mg/g to 25.80 ± 0.12 mg/g, 8.27 ± 0.19 mg/g to 6.68 ± 0.09 mg/g and 165.24 ± 0.58 mg/g to 140.52 ± 0.41 mg/g in phytate, alkaloid and tannins concentrations respectively. Similarly, the concentration of saponin and oxalate significantly ($p < 0.05$) decreased with increasing sprouting time until day four before increase was observed from days 6–7. Fig. 4b (ST-5) shows the % reduction of anti-nutrient in Sorghum bicolor after sprouting. The result revealed that phytate and saponin had the least % reduction (8.93 ± 0.26 and $9.88 \pm 0.0.12$) while the % reduction of oxalate (39.44 ± 0.52) was significantly higher than the other anti-nutrients.

The result in Fig. 4c (ST-6) shows the influence of sprouting time on the anti-nutritional content of Bambara nut flours. It was observed that the concentration of anti-nutrients in the Bambara nut flour significantly ($p < 0.05$) decreased with increase in sprouting time till the fourth (alkaloid, saponin, oxalate and tannin) and fifth (phytate) days but increased afterwards. There was no significant difference ($p < 0.05$) in the values obtained for phytate on days 4 and 5, alkaloid, oxalate and tannins on days 3 and 4 respectively. The percentage reduction of the quantified anti-nutrients in Sorghum after sprouting is presented in Fig. 4d (ST-7). It was observed that tannin was least reduced (9.69 ± 0.11 %) after sprouting compared to oxalate that was reduced by 38.78 ± 0.81 %

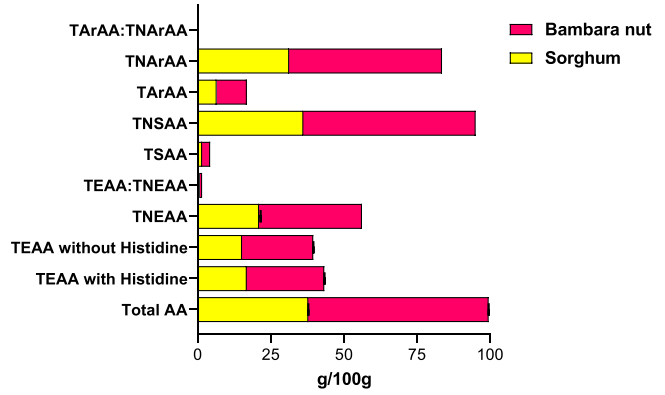

Fig. 3. Amino Acid Classes of Sorghum and Bambara Nut Flour (AA-Amino acid, TEAA-Total essential amino acid, TNEAA-Total non-essential amino acid, TSAA-Total sulphur amino acid, TNSAA-Total non-sulphur amino acid, TArAA-Total aromatic amino acid, and Total non-aromatic amino acid).

3.4. Proximate composition of formulated diets

The proximate composition of the formulated diets (Fig. 5; ST-8) showed that there was a significant difference ($p < 0.05$) in the ash contents of the different diets when supplementation with Bambara flour was increased. There was a statistically significant ($p < 0.05$) increase in the fat content of the diet supplemented with 20 % Bambara. In all the diets the 100 % un-sprouted sorghum diet had the lowest fat content. Protein content of the diets increased with corresponding increase in supplementation with Bambara nut flour. All the diets had significantly different moisture content ($p < 0.05$), although was found to be generally low in all the diets. However, the 100 % sprouted sorghum diet had the highest moisture content while the un-sprouted Bambara diet had the lowest. The crude fibre was found to be lowest in the sprouted Bambara nut diet (100 %) and highest in the un-sprouted sorghum diet. There was no significant difference ($p > 0.05$) in the fibre contents of the 10 % and 15 % Bambara supplemented diets. The carbohydrate content of the un-sprouted sorghum diet was significantly ($p < 0.05$) higher compared to all the other diets.

3.5. Functional properties of sprouted and unsprouted sorghum and Bambara nut flours and sprouted formulated diets

Fig. 6 (ST-9) presents the functional properties of unsprouted and sprouted Bambara nut flour while Fig. 6 (ST-10) shows the functional properties of unsprouted and sprouted sorghum flour. The gelatinization temperatures for Bambara nut and sorghum flours decreased significantly ($p < 0.05$) after sprouting the samples. Conversely, sprouting increased the gelatinization time for Bambara nut but had no effect on the gelatinization time of sorghum flour. Sprouting had no effect on swelling capacity on the two seeds, either sprouted or unsprouted. The same trend was observed for water absorption capacity of the flours. On the other hand, sprouting reduced the bulk densities of Bambara and sorghum flours significantly ($p < 0.05$). There was no observable effect in the foam capacity of sorghum after sprouting, but that of Bambara nut was significantly ($p < 0.05$) reduced upon sprouting.

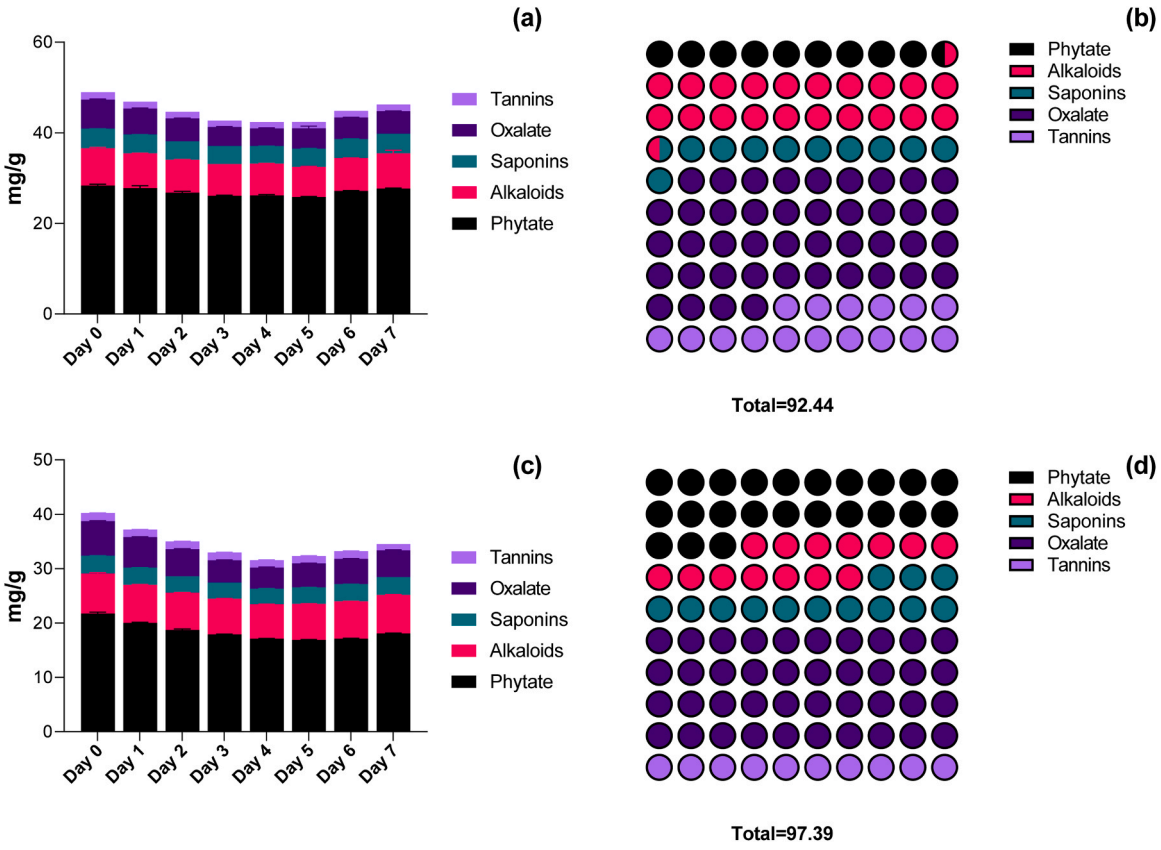

Fig. 4. Anti-nutrient composition of sorghum flour sprouted (a) and Bambara nut flour sprouted (c) at varying durations; and percent reduction of Anti-nutrient in Sorghum bicolor (b) and in Bambara nut (d).

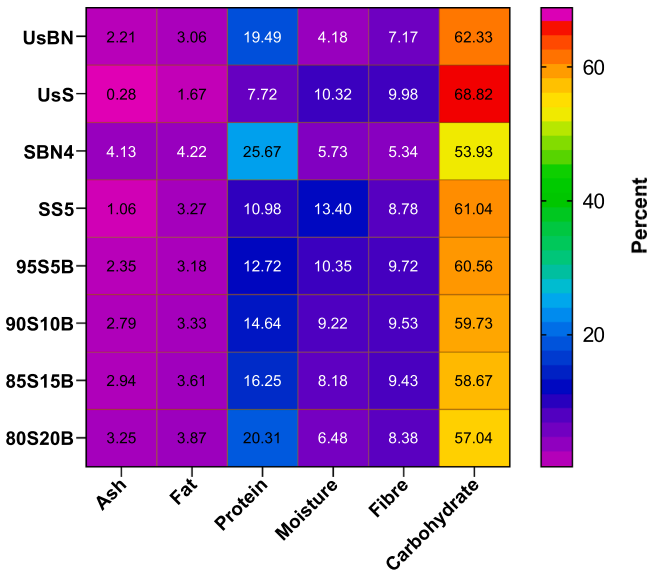

Fig. 5. Proximate Composition of Sprouted, Un-sprouted Bambara Nut and Sorghum Formulated Diets (%). [UsBN: Un-sprouted Bambara Nut, UsS: Un-sprouted Sorghum, SBN4: Sprouted Bambara at day 4, SS5: Sprouted Sorghum at day 5, 95S5B: 95 % Sorghum + 5 % Bambara, 90S10B: 90 % Sorghum + 10 % Bambara, 85S15B: 85 % Sorghum + 15 % Bambara, 80S20B: 80 % Sorghum + 20 % Bambara].

The result in Fig. 6 (ST-11) presents the functional properties of sprouted Bambara nut and sprouted sorghum formulated diets. The gelatinization temperatures of the different diets reduced significantly

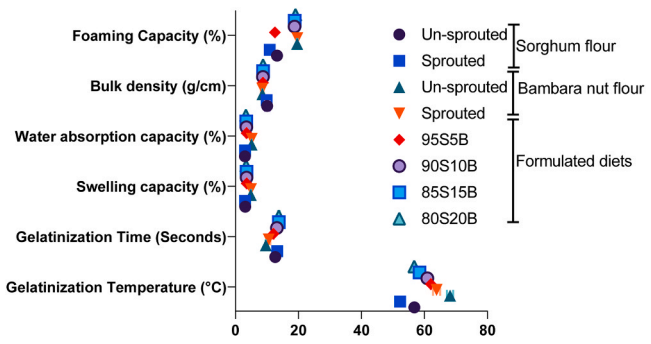

Fig. 6. Functional properties of un-sprouted and sprouted Sorghum flour, Bambara nut flour and formulated diets. [95S5B: 95 % Sorghum + 5 % Bambara, 90S10B: 90 % Sorghum + 10 % Bambara, 85S15B: 85 % Sorghum + 15 % Bambara, 80S20B: 80 % Sorghum + 20 % Bambara].

($p < 0.05$) as the supplementation with Bambara was increased. Also, gelatinization time increased significantly ($p < 0.05$) when supplementation was increased. There was no significant difference ($p > 0.05$) in the swelling capacity of the formulated diets, except for the (80 % sorghum + 20 % Bambara). The water absorption capacity reduced significantly ($p < 0.05$) in the formulated diets as supplementation with Bambara increased. There was no significant difference ($p > 0.05$) in the bulk densities of the formulated diets. Foaming capacity was increased significantly ($p < 0.05$) with a corresponding stability as supplementation with Bambara was increased above 5 %.

4. Discussion

The proximate compositions of sprouted sorghum and Bambara nut as shown in Fig. 1 indicate that sprouting improved some of their nutrient's content. The significant increase ($p < 0.05$) in ash, fat, moisture and protein contents from 4 to 5 days is in agreement with the report of Lemmens et al. (2019) that sprouting times of between 4 and 5 days are needed to maximize nutrient availability in grains and legumes. Nutrient availability during sprouting is attributable to many factors. Metabolic enzymes such as proteinases are activated leading to release of some amino acids and peptides synthesis and utilization of these amino acids may form new proteins (Devi et al., 2015). Sugars and vitamin B-complex are released, minerals are liberated, phenols and polyphenolic constituents in the food which provides anti-oxidants that helps to protect the body against reactive oxygen species are increased. Also, anti-nutritional factors and inhibitors such as phytate, oxalate etc. are reduced remarkably by the action of soaking and sprouting (Nyau et al., 2017; James et al., 2018).

Ash content in food is a measure of its mineral content (Afify et al., 2011). The range obtained in this study for sprouted sorghum is lower than that reported by Raihanatu et al. (2011)) and that obtained for Bambara nut is higher than that reported by Okafor et al. (2014). This difference could be attributed to the source of seeds, soil type, type of fertilizers and climatic condition (Sharif et al., 2014). The observable increase in the ash contents of sorghum and Bambara with sprouting is in agreement with the report of El-Adawy (2002) that sprouting increased the ash content of Mungbam, pea and lentil seeds significantly ($p < 0.05$). The increase in the fat content of the sprouts (sorghum and Bambara nut) is in agreement with the findings of Onwuka et al. (2009) that sprouting increased the fat content of fluted pumpkin seeds significantly. This however, disagrees with the reports of El-Adawy (2002) and Elegbede (1998) that sprouting decreased the fat content of seeds.

The observable increase in the moisture contents of sorghum and Bambara nut agrees with the work of (Ehirim et al., 2018) that sprouting of *Vigna unguiculata* for 120 h increased the moisture content significantly from 5.63 % to 6.72 %. Murugkar and Jha (2009) also observed that moisture content of soybeans increased from 5.4 % to 56.1 % after 48 h of sprouting time. The reason for the increase in moisture content may be hinged on long duration of soaking which allows seeds to absorb much water needed for sprouting and the prolonged water intake as sprouting progresses by the process of imbibition (Ehirim et al., 2018; Nonogaki et al. 2010).

Proteins are critical to normal functioning of life. The increase obtained in the protein contents of sorghum and Bambara corroborates with the work of Bello et al. (2017) who reported a significant increase in the protein content of fluted pumpkin and sorghum after sprouting. The significant increase in the protein content could be ascribed to enzymatic hydrolysis of insoluble proteins to soluble forms (Echendu et al., 2009), and the release of free amino acids after enzymatic hydrolysis for the synthesis of new proteins and enzyme protein (proteases) (Bau et al., 1997; Bliss, 1975).

The initial decrease in the crude fibre in the sprout suggests that fibre could be a source of energy for the enzymatic process of sprouting. However, the later increase obtained in fibre content as sprouting progressed could be attributed to the development of structural carbohydrates, celluloses and hemicelluloses which are major constituents of the cell walls (Shah et al., 2011). This is supported by the report of Ehirim et al. (2018) that the fibre content of Bambara nut increased significantly ($p < 0.05$) after sprouting for 5 days.

The reduction in the carbohydrate content is expected because during sprouting carbohydrate serve as the predominant source of energy for the enzymatic processes and for growth of the embryo (Vidal-Valverde et al., 2002). The activity of the enzyme β-amylase that hydrolyses the starch into simple carbohydrate is increased during sprouting (Suda et al., 1986). Starch content in cotyledon of the seed is also broken down into smaller molecules such as glucose and fructose so as to provide the energy required for cell division as the seeds mature and grow (Nonogaki et al., 2010; Vidal-Valverde et al., 2002). The reduction in the carbohydrate agrees with that reported by Ehirim et al. (2018) that sprouting reduced the carbohydrate content of cowpea from 64.01 % to 47.87 %.

The richer content of Bambara nut is supported by the reports of Maphosa and Jideani (2017a, 2017b) that legumes have higher amino acid and protein compared to cereal crops and hence can serve as substitutes for meat, milk and egg. Suleiman (2016) reported that Bambara nut is rich in amino acid and could be used to supplement sorghum in the formulation of weaning food. The total amino acid obtained for Bambara nut in this study (61.67 ± 0.015 g/protein) ranged within the recommended dietary allowance (RDA) reported by Ijarotimi and Keshinro (2013) for complementary diets.

Glutamate was found to be the most abundant non-essential amino acid in Bambara nut while cysteine was the least. This is in agreement with the report of Suleiman (2016). The composition of the non-essential amino acid in the Bambara flour in this study is more than that reported by Oyeyinka (2016) for Bambara nut flour used in the formulation of complementary food. The reason for this difference may be as a result of the sprouting employed in this study.

Essential amino acids, also known as indispensable amino acids cannot be synthesized by humans and vertebrates, hence must be taken in diets (Lopez & Mohiuddin, 2020). The quality of dietary protein is a measure of the required essential amino acids that it can provide for growth and maintenance of tissues. The total essential amino acid found in this study for Bambara nut flour (43.05 %) is in the same range with that reported by Hussin et al. (2020) for Bambara nut flour (42.6 %). The reason for this similarity, may be as a result of the same species of seed used or the seed were gotten from the same locality. The concentration of leucine, isoleucine, valine, histidine and threonine found in Bambara nut in the present study is lower than that reported by Suleiman (2016). Methionine was found to be the least essential amino acid which is also in agreement with that reported by Hussin et al. (2020). Huang (2012) reported that llysine is an essential amino acid that is primarily used for protein synthesis and it is a limiting amino acid in most cereals. Deficiency of lysine limits protein synthesis leading to weight loss in infants and children. The concentration of lysine in the Bambara nut used in this study suggests that it can aid protein synthesis and support growth of infants and children.

The sulphur containing amino acids (methionine and cysteine) found in the Bambara nut used in this study is higher than that reported by Suleiman (2016) for Bambara nut flour. Sprouting may have enhanced the sulphur amino acid of Bambara nut in this study. The sulphur containing amino acids are important sources of sulphur in the body. Methionine in the form S- adenosyl methionine is required for transmethylation reactions (Rubin et al., 2007) while cysteine is also important detoxicants of specific substances and an important component of gluthathione an important antioxidant in cells. Methionine is essential for protein synthesis, synthesis of antioxidants and lipotropic compounds like taurine, glutathione, choline, carnitine and S –adenosyl methionine (Kiruthikajothi et al., 2014).

Aromatic amino acid (phenylalanine and tyrosine) was found to be high in Bambara nut. Tyrosine is required for the synthesis of certain hormones such as thyroid hormone, epinephrine and norepinephrine and a pigment, melanin (Fernstrom & Fernstrom, 2007). Bambara nut contained sufficient amount of phenylalanine and tyrosine which can be used to supplement sorghum in the formulation of weaning diet. Bambara nut was also found to be a good source of semi essential amino acid, histidine and arginine. These amino acids are referred to as growth promoting factors because they are not synthesised in sufficient amounts during growth, therefore they are crucial in growth of children, pregnant women and lactating mothers (Chatterjea & Shinde, 2007). Methionine and cysteine were the limiting amino acid found in Bambara nut. This agrees with Suleiman (2016). When the essential amino acid provided in

a diet is low, protein synthesis is limited to the rate of the essential amino acid. Hence the essential amino acid is termed limiting and this can reduce growth and maintenance of the body. Though methionine and cysteine were the limiting amino acids in Bambara nut. Bambara has a higher amount of these amino acid that can be used in the supplement sorghum in the formulation of weaning diet.

The significantly lower anti-nutrient levels obtained in the sprouts compared with the values in the unsprouted in this study as shown in Fig. 4 is an indication that sprouting played a significant role in the reduction of anti-nutrients in sorghum and Bambara nut. Processing especially sprouting has been reported to reduce anti-nutrient contents of foods (Nwadi et al., 2019). The low levels of anti-nutrients suggest that food formulated from these sprouts would be safe for human consumption (Sharma, 2020). The reduction in anti-nutrients for sprouted sorghum and Bambara nut is in agreement with that reported by Modu et al. (2014) who observed a remarkable reduction in anti-nutrient contents of corn flour, Bambara nut, cowpea and groundnut nut.

Physical modifications such as (sprouting, fermentation, cooking among others) have been shown to change and enhance functional properties of food (James et al., 2018). Figure 4.1 has shown that sprouting decreased the gelatinization temperatures of sorghum and Bambara nut resulting in increase in gelatinization time. This is in agreement with the works of Ocheme et al. (2015) who reported decrease in gelatinization temperatures and increase in gelatinization time of sorghum flour upon 3–5 days sprouting. This decrease in gelatinization temperatures leading to increase in gelatinization time of sorghum and Bambara nut in the present study may be attributed to the disruption of the starch crystalline making it require low temperature for gelatinization and concentration of the crystalline extending the gelatinization time (Ocheme et al., 2015). The process by which intermolecular bonds of starch molecules is broken down upon application of heat and water giving room for hydrogen bonding sites to absorb more water is known as gelatinization (Ubwa et al., 2012). The decrease in gelatinization temperatures observed in this study suggests that infant formula prepared from sprouted sorghum and Bambara nut will require less heat to attain gelation (James et al., 2018) and this translates to low energy cost in cooking (Owuamanam et al. 2014). Low cooking temperatures helps retain some nutrients in food. Water-soluble vitamins like vitamin C and the B vitamins thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9), and cobalamin (B12) and fat-soluble vitamins like vitamins A, D, E, and K are retained at low cooking temperature. Also, minerals: primarily potassium, magnesium, sodium, and calcium are retained at low temperature (Spritzler, 2017). The decrease in gelatinization temperature of the formulated diets indicates that these nutrients are retained upon cooking. Low temperature and high exposure time have been implicated to promote the availability of the free polyphenols and sugars in food (Alfeo et al., 2020). Gelatinization temperature obtained with extension of cooking time in the preparation of the formulated diets suggests that free polyphenols and sugars in the food will be made available.

In this study sprouting had no effect on swelling capacity of the samples and this is not in agreement with previous reports by Ocheme et al. (2015) and James et al. (2018) that sprouting increased the swelling capacity of sorghum, pearl millet and Bambara nut on day 3. The reason for this variation may be as a result of additional fermentation methods used by these researchers.

The significant reduction in bulk densities of sorghum and Bambara nut is in agreement with the reports of Ocheme et al. (2015), Otutu et al. (2014), Abd Elmoneim and Bernhardt (2010) that bulk densities of seeds reduced significantly after sprouting. The reason for this reduction in bulk is due to the breakdown of the complex compounds such as starch, proteins, and fibres to simple molecule in the course of sprouting (Ocheme et al., 2015). The reduction in bulk observed in this study is advantageous to preparation of weaning foods as foods low in bulk density can be easily digested by infants and weaning children owing to the fact that their digestive system is not fully developed to accommodate bulky foods. Abd Elmoneim and Bernhardt (2010) reported that sprouting is one of the most useful traditional technologies implored in the reduction of bulk in weaning foods.

The decrease in gelatinization temperature and increase in gelatinization time observed for the formulated diets is the same trend as it is for the effect of sprouting on sorghum and Bambara nut flours. The decrease in gelatinization temperature may be an indication that the formulated diets can easily form gel when stirred with boiling water within a short period. Umerah et al. (2020) reported that Gelation capacity indicates the solubility of the native proteins in the continuous phase (water) in the formulated sample.

Water absorption capacity is the index of water absorbed and retained in a sample. The reduction in water absorption capacity obtained upon supplementation of sprouted sorghum with sprouted Bambara in this study is in agreement with the reports of James et al. (2018) that water absorption capacity reduced when sprouted Bambara nut and pearl millet were blended. Low water absorption capacity is required in the preparation of thinner gruels with high caloric density per unit volume. This enhances the absorption of nutrients by infants and reduction in microbial activities as a result of the low water activity leading to extension of the shelf life of the product (Gomez & Aguilera, 1983).

The ability of a formula to foam when water and heat is added is called foaming capacity (James et al., 2018). The increase in foaming capacity may be as a result of supplementation with Bambara nut that is rich in protein. Awuchi et al. (2020) reported that foaming of flours and related foods is attributed to the protein present in the food. Generally, foaming capacity and stability is dependent on the interfacial film formed by the proteins, which maintains the suspension of air bubbles and slows down the coalescence rate.

The observed increase in foam capacity in this study supports the work of James et al. (2018) that reported increase in foam capacity in complementary diet prepared from Bambara nut, sorghum and pearl millets.

The significant increase obtained in the ash content of the formulated diets is due to supplementation with Bambara nut which suggests that the weaning diets are rich in mineral. Afify et al. (2011) reported that ash content is a measure of mineral present in food. The ash content obtained in this study is higher compared to that reported by Suleiman et al. (2016), and Baba et al. (2012) for sorghum fortified with Bambara nut flour. The discrepancies may be due to the quantity and source of the Bambara nut flour used in supplementation in the different studies.

The increase in fat content in this study is in agreement with the work of Suleiman et al. (2016), who observed increase in fat content upon fortification of sorghum with Bambara. The similarity is expected as the same processing method and the same species of Bambara nut was used. The protein enrichment obtained in the formulation of 20 % Bambara supplemented diet is higher than the (RDA) requirement reported by Modu et al. (2014). The remarkable enhancement in the protein content suggests that it can serve as a supportive diet for growing infants and weaning children. Maphosa and Jideani (2017a, 2017b) reported that legumes are excellent source of protein and the use of legumes in supplementation is preferred to animal protein because they contain less fat. Similar report has been published by Oyeyinka (2016) that supplementation with Bambara nut increased the protein of complementary foods.

Low moisture content in food is advantageous to its storage (shelf-life) and also prevent it from microbial attack that can quicken its spoilage (Danso et al., 2019). The moisture content obtained in this study for the formulated diets were lower than that reported by Ijarotimi and Keshinro (2013) for complementary food fortified with African locust bean and Bambara nut but higher than that reported by Suleiman (2016) for sorghum fortified with Bambara nut flour. The reason for this difference may be due to the processing techniques used by the individual researchers.

The fibre content of the supplemented diets was higher compared to

the 100 % sorghum flour. This is in agreement with the work of Mesfin and Shimelis (2013) who observed progressive increase in fibre following incorporation of soybean flour into cereal based bread. The increase in fibre content of the blend in the present study is due to the incorporation of Bambara flour that is high in fibre content. High fibre content in food have been implicated to enhance food quality. Belluco et al. (2013) reported that high fibre content in food is advantageous to bowel movement of food and aid promotion of gut microbes. Anderson et al. (2009) also reported that fibre plays a critical role in the prevention of overweight, constipation in adult and children, cardiovascular disease, diabetes and colon cancer. The reports above suggests that the high fibre content obtained in the present study for the formulated diets may play potential role in bowel movement of food during digestion and would enhance the growth of gut friendly microbes.

The decrease in carbohydrate content of the blends with increase in proportion of Bambara flour supplementation is expected because it follows the trend reported in several studies that supplementation of cereals with legumes reduced carbohydrate content of the cereals. For instance, Bintu et al. (2017) reported a decrease in carbohydrate when he fortified cereals with cowpea and Bambara. Baba et al. (2012) also found a reduction in carbohydrate upon fortification of cereal-based food with cowpea and groundnut. However, the carbohydrate content of the diets found in this study is in the range of the recommended dietary allowance (RDA) reported by Bintu et al. (2017). The high carbohydrate content observed in the present study suggests that the prepared diets are rich in energy and are able to satisfy the energy requirement for infants and weaning children.

5. Conclusion

From this study, Bambara nut and sorghum seeds flour contained both essential and non-essential amino acids as well as macro nutrients, however, Bambara nut may be judged richer especially in protein content than sorghum seed flour. Methionine and cysteine were the limiting amino acids present in sorghum and Bambara nut respectively. Worthy of note is the remarkable enhancement in the protein content of sorghum upon supplementation with 20 % Bambara which suggests that it can serve as a supportive diet for growing children. From the present finding, sprouting sorghum for a duration of five (5) days will allow for an optimum nutritional composition whereas sprouting for four (4) days will optimized the nutrient content of Bambara nut. Upon sprouting, Sorghum and Bambara nut had enriched protein, ash, fibre and fats contents while anti-nutrients decreased significantly below permissible limit. Sprouting also reduced some functional properties the composite diets as these parameters may influence the general acceptability of the diet.

CRediT authorship contribution statement

Ojochenemi Johnpaul Enemali: Methodology, Investigation, Data curation. **Ifeanyi Famous Ossamulu:** Writing – original draft, Methodology, Investigation. **Helmina Olufunmilayo Akanya:** Project administration, Methodology, Conceptualization. **Evans Chidi Egwim:** Supervision, Methodology, Conceptualization. **Naga Raju Maddela:** Writing – review & editing, Software, Formal analysis. **Oluwafemi Adebayo Oyewole:** Writing – review & editing, Validation.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foohum.2025.100722.

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