AMINO ACID PROFILE AND MICROBIOLOGICAL QUALITY OF COMPLEMENTARY DIETS FROM BLENDS OF FERMENTED MILLET (*PENNISETUM GLAUCUM L)*, WITH GROUNDNUT (*ARACHIS HYPOGEAE D*) AND *MORINGA OLEIFERA* FLOURS

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Abstract

Malnutrition resulting mostly from poor feeding practices of infants and young children is a major public health problem in Nigeria. The combinations of two or more plant-based food materials in the preparation of complementary foods in order to improve the nutritional quality have been proven by various scientific studies. This study evaluated the amino acid profile and microbiological quality of flour blends formulated from fermented millet, groundnut and Moringa oleifera. Seven blends were formulated using Nutri Survey for Linear Programming Software and compared with Ogi (traditional complementary food) and cerelac (commercial proprietary). The complementary blends was formulated from different ratio of blends (50:35:15, 50:30:20, 55:25:20, 60:20:20, 70:10:20, 55:20:25, 55:15:30) of fermented millet, groundnut and M. Oleifera. The proximate, amino acids profiles and microbiological qualities were determined using standard methods of analysis. Data were analyzed using statistical packages, means values separated using Duncan's New Multiple Range (DNMR) test at $P<0.05$. Glutamic acid (9.91-13.00 g/100 g) was found to be the most abundant amino acid in the formulations while tryptophan (1.76 – 2.26 g/100 g) was the least concentrated essential amino acids. The results further showed the TAA values (57.02 – 89.11 g/100 g of protein) of formulated samples were found to be higher than value for ogi (61.56 g/100 g of protein) except for FMGM5 (57.02 g/100 g) but lower to compared Cerelac (87.85 g/100 g of protein). total essential amino acids (TEAA) 22.77- 38.81 g/100 g of protein. The mean total viable count of the formulated samples ranged between 1.00×10^2 and 2.50×10^4 cfu/ g, mean mould/yeast count ranged from 1.20×10^1 to 1.80×10^2 cfu/g . However, coliform and Escherichia coli were not detected in the formulated samples indicating they are fit for human consumption. The mean mould/yeas count in all the formulated diets were below the maximum level of 1.0×10^3 recommended by FAO/WHO The findings of this work are an indication that the formulated complementary foods have the potential to provide adequately essential amino acids required by infants for growth and development. Therefore, use of the combination of millet, groundnut and Moringa Oleifera seeds for the prevention of malnutrition in children should be further explored.

Key words: Millet, Fermentation, Amino acid, Mould, Moringa Oleifera

Introduction

The traditional complementary foods use in Nigeria as well as other developing countries is commonly prepared from cereals such as maize, millet, sorghum (Ikujenola and Ogunba, 2018). They are characterized by low protein, low energy density and high bulk (Oyarekua, 2014; Ajibola *et al.,* 2016) and do not supply the essential amino acids (lysine and tryptophan) in adequate amount to meet the nutritional needs of infants (Zakari *et al.,* 2018). The traditional complementary foods have been implicated in the etiology of Protein-Energy-Malnutrition (PEM) among children especially during weaning period (Ikujenola and Ogunba, 2018). Several evidence has revealed that Protein-Energy Malnutrition constitutes a serious nutritional and health problem for children between 6 to 24 months of age in developing countries including Nigeria, most due to poor infant and young child

feeding practices, low protein quality commonly associated with plant-based single diets as well as nutrient losses during processing (Addis *et al.,* 2013). This nutritional deficiency results in growth retardation, increase in morbidity and mortality rate among children particularly among the low-income families who fortified proprietary complementary foods is beyond their reach due to high cost such families often depend mainly on cereals porridge made from millet, maize and sorghum (Compaoré, *et al.,* 2011; Kumari *et al.,* 2017). The use of two or more plant-based food materials (cereal and legume) in the preparation of complementary foods in order to improve the nutritional quality of the food combinations have been proven by various scientific studies (Anigo *et al.,* 2010; Ajibola *et al.,* 2016).

Millet is a term used generally for a wide range of cereals that describes seeds from several taxonomically divergent species of grass. Because of its ability to grow under adverse weather conditions such as low rainfall, it is grown mostly in marginal areas under agricultural conditions in which major cereal fail to give substantial yield and is an important food in many developing countries (Yadav *et al.,* 2014). Millet as a cereal is classified with maize, sorghum and coix in the grass sub-family *Panicoideae*. The nutritional composition of millet include starch, protein, lipids, fibre, vitamins, polyphenols and minerals. When compared to other major cereal crops such as wheat, rice and sorghum, pearl millet had higher protein (14.0 %), fat (5.7%), fiber (2.0 %) and ash (2.1 %) content (Awadelkareem *et al.,* 2015). Researches have also shown its superior protein quality in term of tryptophan and threonine content along with higher content of calcium, iron as well as zinc) makes millets very useful for human (Yadav *et al.,* 2014; Awadelkareem *et al.,* 2015).

Groundnut, *Arachis hypogaea* L. also known as peanut or earthnut is grown as an annual crop principally for its edible oil and protein rich kernels seeds, borne in pods which develop and mature below the soil surface (Zakari *et al*., 2018). Groundnuts are very rich in proteins, oils, fibre, vitamins, carbohydrate and are consumed all over the world due to its availability and affordability compared to other types of nuts (Arya *et al.,* 2016). The ranges of Groundnut nutrient constituents reported were protein 20.7%–25.3%, crude fat 31%–46%, ash 1.2%–2.3%, crude fiber 1.4%–3.9%, carbohydrate 21%–37%, and moisture 4.9%–6.8% (Alhasan *et al.,* 2017). The high nutrient and bioactive contents as well as its affordability, makes peanuts a viable option for improving the nutrition status of those who are malnourished, developing, growing, or in need of critical nutrients (Arya *et al.,* 2016; Mupunga *et al.,* 2017).

M. oleifera is known by diverse names in Hausa" Zogale", among the Yorubas "ewe ile" and in English it is refers to as the "miracle tree". The seeds of *M. oleifera* are considered to be antipyretic, acrid, bitter and they possess antimicrobial activity (Gopalan *et al.,* 2007). *M. oleifera* seeds are good source of protein, vitamins, beta-carotene, amino acids and various phenolics (Anwar *et al.,* 2007). *M. oleifera* seeds are eaten as peas or roasted, pounded or pressed into sweet, non-desiccating oil, commercially known as 'Ben oil' of high quality. However, despite its richness in protein, minerals and vitamins, it has been one of the underutilised plant resources (Ijarotimi *et al.,* 2013).

This study evaluated the amino acid profile and Microbiological quality of flour blends prepared from fermented millet, groundnut and *Moringa oleifera*.

MATERIALS AND METHOD

Sources of food materials

Millet (*Pennisetum glaucum*), Groundnut (*Arachis hypogeae*) and *Moringa oleifera* were obtained from the central market Lafia, Nasarawa State capital, Nigeria. The food materials were purchased fresh and of good quality.

Preparation of materials

Pearl millet were cleaned by hand picking and steeped in cold water for 24 hours. It was then washed and spread on a wire gauze on a raised platform. The grains were dried and then hammer milled into powder and sieved to fine powder using a *60mm* mesh sieve. Groundnut were cleaned and then roasted in an oven at 60^0C for 30 minutes and dehulled to obtained clean roasted groundnut. Moringa oleifera seeds flour was prepared according to the method described by Ijarotimi *et al.,* 2013. Moringa oleifera seeds were sorted, dehulled, boiled for 1 hour, wrapped in blanched banana leaves for 72hrs (3days) at room temperature to ferment. then oven dried at 60°C for 20 hrs (model T121, Gen lab Widnes, UK), the fermented seeds were milled using a Philips laboratory blender (HR2811 model), and sieved using a 60-mm mesh sieve (British Standard). The food materials were blended into different formulation of appropriate ratio after which the food samples were milled, sieved and packed in plastic container and stored at room temperature (25^0C) prior to analyses (Fig.1) .

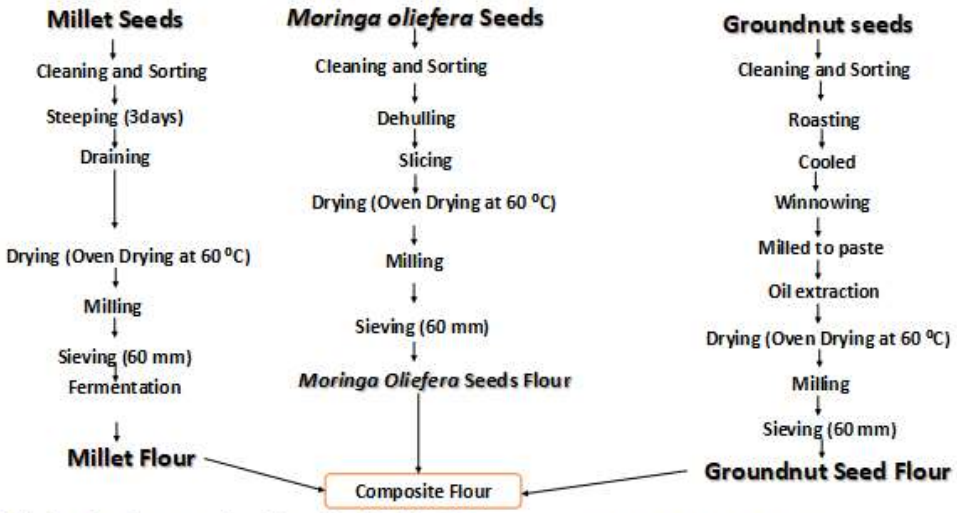


Fig. 1. The flow chart for preparation of Formulated food blend Anigo et al. (2010);; Ijarotimi et al. (2013)

Formulation of food samples

In order to achieve the desired food balancing that meets the calorie and protein requirements of infants between 6 and 24 months of age food formulations were done by blending different components of food samples in the appropriate ratios according to their nutrient contributions. Seven blends were formulated using Nutri Survey for Linear Programming Software and compared with *Ogi* and cerelac. The complementary blends was formulated from different ratio of blends (50:35:15, 50:30:20, 55:25:20, 60:20:20, 70:10:20, 55:20:25, 55:15:30) of fermented millet, groundnut and *M. Oleifera* (Table 1). The Nutri-Survey Linear Programming Software was used to target 18% protein, 9% fat (Amankwah *et al*., 2009) and minimum energy value of 380 kcal/100g dry matter requirement specifications in the weaning blend formulation for the age group of 6 to 24 months.

Table 1: Formulation of samples

Samples	FMGM 1	FMGM2	FMGM3	FMGM 4	FMGM 5	FMGM 6	FMGM7
Pearl Millet(%)	50	50	55	60	70	55	55
Groundnut (%)	35	30	25	20	10	20	15
Moringa Oleifera(%)	15	20	20	20	20	25	30

Key: **FGMG:** Fermented Pearl Millet+ Groundnut + Moringa Oleifera

Determination of proximate composition flour blends

The AOAC (2005) standard method was used to determined nutrients composition of the formulated samples. Oven method was used for moisture determination, fat was determined by ether extraction using soxhlet extractor, crude protein microkjedahl method using 6.25 as a conversion factor while ash was determined by drying ashing method.

The carbohydrate content was determined by difference, that is subtracting the total of %moisture content, % crude protein, %crude fiber, % ash, and % fat from the total dry weight (100 %) of the food sample difference as follows:

Total Carbohydrate (%) =100% – % (Moisture + Protein + Fat + Ash + Crude Fibre) The energy content was determined by the method of The energy values of the food samples was calculated from, carbohydrate, protein and fat content using the Atwater's conversion factor that is the sum of 4.0 kcal/g carbohydrate, 4.0 kcal/g protein and 9.0 kcal/g fat as described by Iombor *et al.,* (2009).

Determination of Amino acid Profile.

The High Performance Liquid Chromatography (HPLC) system method as described by AOAC (2005) was used for the determination of amino acid composition of the formulated samples. The samples were digested and hydrolyzed with 6M Hydrochloric acid (HCL) for 24 hr using the Applied Biosystems Phenylthiohydantoin (PTH) Amino Acid Analyzer. The determination of the concentration of tryptophan was deermined after alkaline hydrolysis as described by Ikujenola and Ogunba (2018) while after acid oxidation was used in the determination of the methionine and cysteine concentrations (Gerhrke *et al.,* 1985). The amino acids were calculated as grams of amino acid per 100g crude protein of food samples.

Determinations of Microbiol*ogi*cal Qualities of Flour Samples :

The microbial analysis was carried out on the food samples as described by Oyarekua (2011). The analyses were carried out in triplicates. The mean colony obtained from the countable triplicate plates, were expressed as colony forming unit per gram (cfu/g).

Statistical analysis. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 for Windows. The mean and standard error of the mean (SEM) of the results were calculated. The comparison of the mean differences in proximate, amino acids, between food groups was determined using one-way analysis of variance (ANOVA). Significant difference was determined at $p<0.05$. Duncan's Multiple Range Test (DMRT) was used to separate the means

Result and Discussion:

The results of the proximate composition of the formulated samples are presented in Table 1. The moisture content of flour samples ranged between 5.50 g/ 100g in FMGM3 to 8.29 g/100g in FMGM5. The moisture content observed the samples were lower than the value (7.8 g/ 100g) observed for '*Ogi*' except in FMGM7 (8.23 g/ 100g). The moisture content for the formulation were observed to be significantly different from each other ($p<0.05$). The values were comparatively similar to the values (7.18 – 7.80 g/100 g) reported by (Famakin *et al.*, 2016) for plantain-based dough meal enriched with soybean and cassava fibre but lower when compared with 11.5 to 14.39 g/ 100g values reported for food gruel formulated from malted cereals, soybeans and groundnut by Anigo *et al.* (2010). Furthermore, the moisture contents of the formulated complementary foods were higher than the FAO/WHO recommended values of less than 5% for infant foods (Table 2). Hence, there may be a need for further drying of the developed food samples to meet the FAO/WHO standard which will completely discourage microbial growth and spoilage chemical reaction thereby prolong the shelf life of the flour products. Low moisture content in food samples is recommended because high moisture encourages microbial growth and thereby reduced the storage quality hence render the diets unsuitable for consumption (Solomon, 2005).

The crude protein content of the formulated food samples ranged from 18.18 (FMGM4) to 20.46 g/100g (FMGM5). The protein values for the formulated samples were significantly higher ($p<0.05$) than cerelac (17.36 g/100g) and *ogi* (7.08 g/100g) respectively and also than the 13 to 14g RDA recommended for infants up to one year (FAO/WHO, 1998). The protein content in the formulated food samples are higher than 1.38 to 3.15g/100g reported by (Anigo *et al.,* 2010). Studies have shown that fermentation processing techniques usually improved the nutrient quality (i.e., protein content) (Rasane *et al.,* 2015; Ajibola *et al.,* 2016; Kumari, 2017). The high crude protein values observed in this study may be attributed to the supplementation of flour from pearl millet with other protein rich source such as groundnut and Moringa oleifera seed flour as affirmed by (Shiriki *et al*., 2015). This result confirms observations from several authors showing that the protein content of two or more plant-based food materials, particularly cereal-legume combinations, are usually better than those produced from a single cereal or other plant based food materials (Shiriki *et al.,* 2015; Gopalakrishnab *et al.,* 2016).

Energy values of the samples ranged from 427.14±2.72 Kcal/100 g in FMGM2 to 481.16±0.38 Kcal/100 g in FMGM6. The results showed that the energy density per 100 g were lower than the minimum energy (483.9 Kcal/ 100g) recommended in the Codex Alimentarus standards for weaning /followup foods (WHO/FAO,1998) but comparable with the energy contents of *Ogi*' and *Cerelac* which are more energy dense (423.93 and 434.71 Kcal/100 g). This high-energy content of the formulated complementary food samples observed in this study may be due to the high content of carbohydrates and lipids. According to FAO/WHO (1998), foods fed to infants and children should be energy dense because low-energy foods tend to limit total energy intake and the utilisation of other nutrients.

The amino acid compositions of the formulated samples are presented in Table 3. The most abundant amino acid in the formulations is glutamic acid (9.91-13.00 g/100 g) while the least (1.76 – 2.26 g/100 g) concentrated essential amino acids is tryptophan. The results showed that total amino acids (TAA) ranged from 57.02±0.01-89.11±0.05 g/100 g of protein, total essential amino acids (TEAA) 22.77- 38.81 g/100 g of protein, % TEAA/TAA ranged from 38.39 - 44.38%. The results further indicated that the TAA values (57.02 – 89.11 g/100 g of protein) of formulated samples were higher than value (61.56 g/100 g of protein) obtained for ogi except for FMGM5 (57.02 g/100 g) and when

compared to the value (87.85 g/100 g of protein) for Cerelac they were lower except for FMGM1 (89.11 g/100 g of protein) which was higher. This observation agreed with other findings that a single plant-based food, such as Ogi (corn gruel), is usually low in essential amino acids but when combined with legume or other protein-rich food materials, the nutritional quality of the combinations usually improved and better than unfortified single plant-based food product (Omueti *et al*., 2009: Ajibola *et al*., 2016).

The percentage ratio of TEAA to TAA in the formulated complementary foods were in the range of 38.- 44% which is above the 36% considered adequate for ideal protein for infants (FAO/WHO/UNU, 2005; Esan *et al.,* 2018). The findings is an indication that the formulated complementary foods have the potential to provide adequately the essential amino acids required by infants for their growth and development. The detection of histidine and arginine (important essential amino acids for children) in high concentration in the complementary foods is also an added advantage because the metabolic pathways that synthesize these amino acids are not fully developed in children (Solomon, 2005).

The result of microbial composition of the formulated diets are shown in Table 4. The mean total viable count of the formulated samples ranged between 1.00 x 10^2 and 2.50 x 10^4 cfu/ g, mean mould/yeast count ranged from 1.20 x 10^1 to 1.80 x 10^2 cfu/g . However, coliform and Escherichia coli were not detected in the formulated samples. This shows that both are fit for human consumption. The total viable count and *E. coli* in all the formulated diets were found to be below the maximum level of 1.0 x10^3 recommended by FAO/WHO (FAO/WHO, 1998)

Conclusion and Recommendations

The findings of this work are an indication that the formulated complementary foods have the potential to provide adequately the essential amino acids required by infants for the growth and development. The percentage ratios of TEAA to TAA in the formulated complementary foods were in the range of 38.-55% which is above the 36% considered adequate for ideal protein for infants. Therefore, use of the combination of millet, groundnut and Moringa Oleifera seeds for the management of malnutrition in children should be further explored.

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Table 2: Proximate compositions (g/100 g dry weight matter) and energy value of complementary foods from fermented Millet, Groundnut and *Moringa oleifera* blends

Nutrient	FMGM 1	FMGM 2	FMGM3	FMGM 4	FMGM 5	FMGM 6	FMGM 7	*CERELAC*	*OGI*	*RV(g)
Moisture	7.40^{bc} ±0.06	7.00^{c} ±0.12	5.50^{d} ±0.06	7.07^{c} ±0.03	8.23^{a} ±0.38	7.00^{c} ±0.06	6.97^{c} ±0.09	4.03^{e} ±0.01	7.80^{ab} ±0.12	<5
Total Ash	2.71^{b} ±0.06	2.68^{b} ±0.06	1.62^{d} ±0.09	2.60^{b} ±0.06	2.38^{c} ±0.09	3.30^{a} ±0.07	3.30^{a} ±0.07	2.51^{b} ±0.01	1.18^{e} ±0.01	<3
Crude Fat	11.73^{d} ±0.32	10.89^{e} ±0.52	19.28^{a} ±0.09	17.57^{c} ±0.03	19.04^{b} ±0.07	20.72^{a} ±0.03	18.96^{ab} ±0.07	9.87^{f} ±0.01	5.61^{g} ±0.01	10 - 25
Crude Protein	20.18^{a} ±0.07	18.46^{b} ±0.09	19.85^{a} ±0.03	20.46^{a} ±0.07	18.18^{c} ±0.07	18.41^{b} ±0.09	18.38^{b} ±0.06	17.36^{c} ±0.01	7.08^{d} ±0.12	16
Crude Fibre	3.54^{cd} ±0.06	3.58^{cd} ±0.07	3.89^{b} ±0.06	3.72^{c} ±0.03	3.44^{d} ±0.12	3.58^{cd} ±0.07	3.51^{cd} ±0.07	5.43^{a} ±0.12	0.92^{e} ±0.01	10 -15
CHO	61.53^{d} ±0.13	63.83^{c} ±0.59	55.23^{e} ±0.03	56.63^{e} ±0.10	56.92^{e} ±0.20	55.26^{f} ±0.06	55.88^{ef} ±0.067	69.11^{b} ±0.12	86.28^{a} ±0.12	64
Energy (Kcal)	432.14^{f} ±2.17	427.17^{f} ±2.72	473.84^{a} ±0.55	466.49^{d} ±0.12	471.76^{cd} ±1.33	481.16^{b} ±0.38	467.68^{bc} ±0.18	434.71^{e} ±0.61	423.93^{e} ±1.03	400-425

Means (±SEM) with different alphabetical superscripts in the same row are significantly different

FMGM1: 50%Fermented Millet +35%Groundnut + 15%*Moringa oleifera,* FMGM2: 50%Fermented Millet +30%Groundnut + 20%*Moringa oleifera*, FMGM3: 55%Fermented Millet +25%Groundnut + 20%*Moringa oleifera*, FMGM4: 60%Fermented Millet +20%Groundnut + 20%*Moringa oleifera*, FMGM5: 70%Fermented Millet +10%Groundnut + 20%*Moringa oleifera*, FMGM6: 55%Fermented Millet +20%Groundnut + 25%*Moringa oleifera;* FMGM7: 55%fermented Millet +15%Groundnut + 30%*Moringa oleifera*, *CODEX CAC/GL.08, 1998

Table 3: Amino Acid compositions (g/100g dry weight matter) of complementary foods from fermented Millet, Groundnut and *M. oleifera* flour blends

Nutrient	FMGM 1	FMGM 2	FMGM 3	FMGM4	FMGM 5	FMGM 6	FMGM 7	CERELAC	OGI	*Children
Non-Essential Amino Acid										
Alanine	5.81^{a} ±0.012	4.33^{d} ±0.12	5.55^{b} ± 0.03	5.01^{c} ±0.00	4.02^{e} ±0.00	3.95^{e} ±0.12	4.02^{e} ±0.00	1.40^{g} ±0.03	3.00^{f} ±0.03	
Aspartic acid	7.01^{b} ±0.01	6.33^{e} ±0.01	6.62^{c} ±0.01	6.20^{d} ±0.01	4.03^{h} ±0.01	4.65^{g} ±0.01	6.27^{e} ±0.12	9.23^{a} ±0.04	5.97^{f} ±0.07	
Serine	4.60^{b} ±0.01	5.00^{a} ±0.01	4.00^{d} ±0.00	3.60^{e} ±0.12	2.54^{h} ±0.12	2.88^{g} ±0.01	3.07^{f} ±0.06	4.31^{c} ±0.01	4.60^{b} ±0.08	
Glutamic acid	12.62^{d} ±0.12	13.00^{c} ±0.01	12.83^{cd} ±0.00	12.64^{e} ±0.02	11.00^{f} ±0.12	10.67^{g} ±0.12	9.91^{h} ±0.06	19.65^{a} ±0.05	16.95^{b} ±0.03	
∑NEAA	30.04±0.01	28.66±0.04	29.00±0.01	27.45±0.04	21.59±0.06	22.05±0.04	23.27±0.01	34.59±0.03	30.52±0.05	
Conditionally essential amino acid										
Proline	4.20^{a} ±0.06	3.30^{c} ±0.12	3.80^{a} ±0.00	2.00^{g} ±0.12	2.26^{f} ±0.12	2.93^{d} ±0.07	3.30^{c} ±0.01	3.35^{b} ±0.03	2.55^{e} ±0.01	
Glycine	3.32^{a} ±0.12	1.64^{c} ±0.01	2.55^{b} ±0.12	1.42^{e} ±0.00	1.05^{f} ±0.12	2.04^{d} ±0.01	2.37^{b} ±0.12	5.97^{f} ±0.07	4.12^{g} ±0.02	
Arginine	7.23^{b} ±0.12	6.03^{d} ±0.01	6.89^{c} ±0.01	5.34^{e} ±0.01	5.30^{e} ±0.29	5.90^{d} ±0.01	6.03^{d} ±0.01	9.13^{a} ±0.07	4.80^{f} ±0.00	
Cysteine	2.41^{a} ±0.12	2.20^{c} ±0.12	2.26^{b} ±0.01	2.10^{d} ±0.07	1.82^{f} ±0.12	1.82^{f} ±0.01	1.95^{e} ±0.00	1.10^{g} ±0.07	0.82^{h} ±0.00	
Tyrosine	3.10^{c} ±0.06	2.75^{d} ±0.01	3.30^{b} ±0.00	3.10^{c} ±0.01	2.23^{g} ±0.02	2.60^{e} ±0.01	2.80^{d} ±0.01	2.33^{f} ±0.02	4.16^{a} ±0.02	
∑CEAA	20.26±0.10	15.92±0.05	18.80±0.03	13.96±0.04	12.66±0.13	15.29±0.02	16.45±0.03	21.88±0.05	16.45±0.01	
Essential amino acid										
Lysine	3.02^{b} ±0.01	2.52^{c} ±0.12	2.70^{c} ±0.00	2.31^{d} ±0.12	2.17^{de} ±0.01	2.02^{ef} ±0.01	2.30^{d} ±0.06	4.09^{a} ±0.07	0.51^{f} ±0.00	6.4
Threonine	4.40^{a} ±0.12	4.01^{c} ±0.01	4.22^{b} ±0.01	3.67^{d} ±0.01	2.94^{e} ±0.01	3.05^{e} ±0.01	3.67^{d} ±0.12	3.83^{cd} ±0.01	1.75^{f} ±0.00	3.7
Valine	5.62^{a} ±0.12	5.33^{a} ±0.32	5.67^{a} ±0.12	4.68^{b} ±0.01	3.33^{d} ±0.01	3.60^{d} ±0.01	4.01^{c} ±0.01	4.72^{b} ±0.06	1.86^{e} ±0.02	3.8
Methionine	2.30^{c} ±0.01	2.10^{d} ±0.01	2.20^{c} ±0.12	2.02^{e} ±0.01	1.71^{f} ±0.01	1.71^{e} ±0.00	1.76^{e} ±0.01	1.71^{b} ±0.06	1.10^{a} ±0.00	2.7
Isoleucine	4.91^{a} ±0.01	3.02^{d} ±0.01	4.00^{c} ±0.00	2.92^{e} ±0.01	2.19^{g} ±0.01	2.36^{f} ±0.01	3.01^{d} ±0.01	4.34^{b} ±0.07	1.33^{h} ±0.02	3.1
Leucine	8.53^{a} ±0.01	7.55^{c} ±0.01	6.59^{d} ±0.01	7.90^{b} ±0.01	3.97^{f} ±0.04	4.70^{e} ±0.01	4.56^{e} ±0.00	4.57^{e} ±0.16	3.51^{g} ±0.02	7.3
Phenylalanine	5.15^{a} ±0.01	4.26±0.01^{d}	4.61^{c} ±0.01	3.90^{e} ±0.00	3.01^{h} ±0.00	3.30^{g} ±0.12	3.02^{h} ±0.00	4.76^{b} ±0.01	3.55^{f} ±0.00	6.9
Histidine	2.62^{a} ±0.01	2.34^{abc} ±0.32	2.50^{ab} ±0.01	2.23^{bcd} ±0.01	1.69^{f} ±0.01	1.92^{def} ±0.12	1.85^{ef} ±0.00	2.11^{cde} ±0.01	0.24^{abc} ±0.00	1.0
Tryptophan	2.26^{b} ±0.12	2.05^{cde} ±0.01	2.21^{bcd} ±0.00	1.81^{f} ±0.12	1.76^{f} ±0.12	1.92^{ef} ±0.00	2.04^{de} ±0.01	1.25^{bc} ±0.00	0.74a±0.01	1.25
TEAAs	38.81	33.18	34.70	31.44	22.77	24.58	26.40	31.38	14.59	
∑AA	89.11	74.76	82.5	73.85	57.02	64.02	67.11	87.85	61.56	
(∑EAA/∑AA)100	43.61	44.38	42.06	42.57	39.93	38.39	39.79	43.63.	23.7	36*

Means (±SEM) with different alphabetical superscripts in the same row are significantly different at P< 0.05 FMGM =Fermented millet + groundnut + *Moringa oleifera* .Key: FMGM1 50%:35%:: 15%, , FMGM2 50%:30%: 20%,, FMGM3 : 55%:25%: 20%, FMGM4: 60%:20%: 20%, FMGM5 70%:10%: 20%,, FMGM6 55%:20%: 25%,FMGM7 55%:15%: 30%, *TEAA: Total Essential amino acid,* ∑AA: Total amino acid, ∑NEAA: Total non essential amino acid, ∑CEAA: Conditionally essential amino acid
*Recommended value (5)

Table 4: Microbial count of fermented, Millet, Groundnut and *Moringa oleifera* blends

SAMPLES	*Escherichia coli* (cfu/g)	Total Viable count (cfu/g)	Mould/yeast (cfu/g)	Coliform (cfu/g)
FMGM 1	Nil	1.3×10^2	1.2×10^2	**Nil**
FMGM 2	Nil	1.1×10^2	1.2×10^1	**Nil**
FMGM3	Nil	2.5×10^4	1.0×10^2	**Nil**
FMGM 4	Nil	1.0×10^2	1.3×10^1	**Nil**
FMGM 5	Nil	1.3×10^2	1.3×10^2	**Nil**
FMGM 6	Nil	1.2×10^2	1.8×10^2	**Nil**
FMGM 7	Nil	1.3×10^2	1.1×10^2	**Nil**
CEREAL	Nil	1.1×10^2	1.1×10^2	**Nil**
OGI	Nil	1.6×10^2	1.1×10^2	**Nil**
*Acceptable level	0	$<10^3$	$<10^2$	**0**

[Nill] Not detected; FMGM :Fermented millet + groundnut + *Moringa oleifera* .Key: FMGM1 : 50%:35%:: 15%, , FMGM2 : 50%:30%: 20%,, FMGM3 : 55%:25%: 20%, FMGM4:: 60%:20%: 20%, FMGM5: 70%:10%: 20%,, FMGM6: 55%:20%: 25% ,FMGM7 : 55%:15%: 30%,