Chemical Compositions and Nutritional Properties of Popcorn-Based Complementary Foods Supplemented With *Moringa oleifera* Leaves Flour

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Abstract

Cereal gruel is the common complementary foods in developing countries, and it is usually low in energy and protein; hence, responsible for increase in protein-energy malnutrition among underprivileged weaning aged children. Several locally available food materials have been tested in combination for infant food formulations however; popcorn and *Moringa oleifera* leaves combination have not been used. After blanching and fermentation processing, popcorn and moringa leaves were milled into flour and blended to obtain, blanched popcorn-moringa leaves (BPM) (65% popcorn and 35% moringa leaves flour) and fermented popcorn-moringa leaves (FPM) (65% popcorn and 35% moringa leaves flour). Products were analyzed for chemical composition, functional properties and bioassay using standard methods. Protein content of FPM (21.27 ± 0.20 g/100 g) and BPM (15.99 ± 0.14 g/100 g) were higher than *Cerelac* (15.75 ± 0.01 g/100 g) and 'Ogi' (6.52 ± 0.31 g/100 g); while energy values of FPM (393.94 ± 0.39 kcal) and BPM (389.69 ± 1.40 Kcal) were lower than 'Ogi' (418.08 ± 0.47 kcal) and *Cerelac* (431.58 ± 0.01 kcal). Mineral contents of BPM were higher in zinc, iron, potassium, sodium and phosphorous, while FPM sample was higher in copper, calcium and magnesium, and were lower than *Cerelac*. Oxalate, phytate and trypsin inhibitor in FPM were lower than BPM. Biological value and protein efficiency ratio of FPM were higher than BPM and 'Ogi', but lower than *Cerelac*. The albino rats fed with the FPM had higher growth rate when compared with those rats fed with BPM sample and 'Ogi', but lower than those fed with *Cerelac*. Nutrient composition and nutritional profile of popcorn-moringa leaves based complementary foods could be used as substitutes for local complementary foods, which are low in protein and energy.

Keywords: popcorn, *Moringa oleifera* leaves, complementary foods, nutritional profile

1. Introduction

Adequate infant and young child nutrition involves adoption of recommended breastfeeding and complementary feeding practices and access to the appropriate quality and quantity of foods (PAG, 1972). Optimal nutrition in the first year of life is therefore crucial in laying the foundation of good nutrition and health of children. Nutritionally, it has been proven that breast milk is a complete and perfect food for the infant during the first six months of life (Lutter & Rivera, 2003). After 6 months breast milk alone can no longer be sufficient both in terms of quantity and quality to meet the nutritional requirements of infants, hence, appropriate complementary foods should be introduced (UNICEF, 2009).

In developing countries including Nigeria, many families cannot afford commercial complementary foods to wean their infants due to high level of poverty, and thereby engage in weaning the children on cereals gruels (Ikpeme-Emmanuel et al., 2012). Scientific findings have shown that cereal gruels are the common complementary foods in developing countries, which is characterized by low energy and protein density (Hotz & Gibson, 2001; Lutter & Rivera, 2003; Inyang & Zakari, 2008; Igyor et al., 2011) due to large volume of water relative to its solid matter contents during preparation. To increase the energy density of the gruel, more of the solid matter are needed, which will makes the gruel too thick and viscous for infant to eat and too large for their stomach capacity (Ikujenlola & Fashakin, 2005). Infants are therefore unable to fulfill their energy and other essential nutrients requirements (Ikujenlola & Fashakin, 2005), hence, there is increased in protein-energy

malnutrition among weaning aged children.

Improved complementary feeding and breastfeeding practices are essential to achieve the Millennium Development Goals (MDGs) for child survival and prevention of protein-energy malnutrition (Lutter & Rivera, 2003). To achieve this goal, formulation and development of nutritious complementary foods from local and readily available raw food materials have received a lot of attention in many developing countries (Plahar & Annan, 1994), however, locally available popcorn and moringa leaves combination have not been tested for complementary foods.

Cereals are widely utilized as food in African countries than in the developed world (Makinde & Ladipo, 2012). For instance, cereals account for as much as 77% of the total caloric consumption in African countries (Mitchel et al., 1997) and contribute substantially to dietary protein intake in a number of these countries. Compositionally cereals consist of carbohydrate and less in amount of protein and essential minerals. Among the cereals, popcorn (a derived product) contains appreciable amount of protein and minerals when compared with other maize species (Iken & Amusa, 2010). Popcorn, which is grown solely for human consumption in the developed countries, is now becoming popular in Nigeria (Iken & Amusa, 2010).

Moringa oleifera, also known as drumstick originated and widely cultivated in India and it has become naturalized in many locations in the tropics (Fahey, 2005). Moringa is one of the newly discovered vegetable and is gaining wide acceptance as medicine and food in Nigeria (Bamishaiye et al., 2011; Ijeomah et al., 2012). *Moringa oleifera* is the most known and widely cultivated variety of the genus *Moringa,* family *Morigaceae* (Fugile, 2001). *Moringa oleifera* is also known by other common names like Mallungay (Philippines), Benzolive tree (Haiti), Horse raddish tree (Florida), Nebeday (Senegal), Zogale (Hausa), Okwe Oyibo (Igbo), ewé ìgbálè (Yoruba) and Jeghel-agede in Tiv (Fahey, 2005; Bamishaiye et al., 2011). The leaves, seeds and flowers all have good nutritional and therapeutic values, and study has shown that the leaves were used to prevent or treat protein-energy malnutrition and other nutritional related diseases (Tete-Benissan et al., 2012). *Moringa oleifera* leaves are low in fat and carbohydrate but are excellent sources of amino acids (Rajangam et al., 2001), particularly sulphur containing amino-acids, that is, methionine and cystine which are often in short supply in cereals and other plant-based foods (Liu et al., 2007).

Several local cereal-legumes based and commercial complementary foods are available in Nigeria, however, locally available popcorn and *Moringa oleifera* combinations have not been used for infant food. This study was therefore designed to formulate and evaluate nutritional qualities of complementary foods using popcorn and moringa leaves flour.

2. Materials and Methods

2.1 Collection of Food Materials

Popcorn kernels were purchased from Erekesan market, Akure while *Moringa oleifera* leaves were obtained from a botanical garden in Akure, Ondo State, Nigeria.

2.1.1 Processing of Popcorn and *Moringa oleifera* Leaves Flour

Fermented popcorn (*Zea mays averta*) flour: The popcorn kernels were sorted, soaked in hot water for 2 days, washed, wet milled using attrition mill, sieved, fermented for 3 days, decanted, oven dried in hot air oven at a temperature of 60 ºC for 2 days, dry milled using attrition mill, sieved through 0.4 mm wire mesh, packed in a sealed air tight plastic container and stored at room temperature prior to formulations and chemical analysis.

2.1.2 Blanched *Moringa oleifera* Leaves Flour

The *Moringa oleifera* leaves were sorted, washed with distilled water, steam blanched, oven dried at a temperature of 50 ºC, milled using a laboratory blender, sieved through a 0.4 mm wire mesh and stored in airtight container at room temperature prior to formulations and chemical analysis.

2.1.3 Fermented *Moringa oleifera* Leaves Flour

The leaves were sorted, washed with distilled water, steam blanched, tightly wrapped in banana leaves to ferment for 72 hours, oven dried in hot air oven at a temperature of 50 ºC for 3 days, milled using laboratory blender, sieved through 0.4 mm wire mesh, tightly packed in a sealed plastic container and stored at room temperature prior to formulations and chemical analysis.

2.1.4 Food Formulations

Proportions of popcorn flour and *Moringa oleifera* leaves flour in the formulations were determined using NutriSurvey-Linear-Programming Software to obtain the following combinations: Blanched Popcorn-*Moringa*

oleifera leaves flour (BPM) (65% popcorn, 35% *Moringa oleifera* leaves flour) and Fermented Popcorn-Moringa leaves flour (FPM) (65% popcorn, 35% *Moringa oleifera* leaves flour).

2.2 Chemical Analysis

2.2.1 Proximate Analysis

Nutrient composition of the food sample was determined in triplicate using the standard procedures of Association of Official Analytical Chemists - AOAC (2005). Five grams of each formulated complementary foods were used to determine the moisture content in a hot-air circulating oven (Galenkamp). Ash was determined by incineration of 2 grams each of the food samples in a Gallenkamp muffle furnace at 550 ºC (Gallenkamp, size 3) (Method No 930.05) [AOAC, 2005]. Crude fat was determined by exhaustively extracting 10 grams of each sample in petroleum ether (boiling point, 40 to 60 ºC) in a Soxhlet extractor (Method No 930.09). Protein (N × 6.25) was determined by the Kjeldhal method (Method No 978.04) using 0.5 gram each of the formulated complementary foods (AOAC, 2005). Crude fiber was determined after digesting 5 grams each of fat-free complementary food samples in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (Method No 930.10) (AOAC, 2005).

2.2.2 Carbohydrate Determination

The carbohydrate content was determined by subtracting the summed up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100 g of the sample [100%-(moisture% + protein % + fat % and ash %)] (Otitoju, 2009).

2.2.3 Gross Energy

Energy was determined by calculation from fat, carbohydrate and protein content using the Atwater's conversion factor; 4.0 kcal/g for protein, 9.0 kcal/g fat and 4.0 kcal/g for carbohydrate (Iombor et al., 2009).

2.2.4 Minerals Determination

AOAC (2005) methods were used to determine mineral compositions of the samples. One gram of sample was digested with nitric/perchloric/sulphuric acids mixture in ratio 9:2:1 respectively, filtered and the filtrate in a 5 ml volumetric flask was loaded to Atomic Absorption Spectrophotometer, (model703 Perkin Elmer, Norwalk, CT, USA). The standard curve for each mineral (calcium, magnesium, iron, aluminum, lead, copper and zinc), was prepared from known standards and the mineral value of samples estimated against that of standard curve. Sodium and potassium values were determined using Flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK). The phosphorus was determined using Vanodo-molybdate method.

Procedure: To series of 100 ml volumetric flasks 0.0, 2.5, 5.0, 7.5, 11.0, 15.0, 20.0, 20.0, 40.0, 50.0 ml of the standard phosphate solution was made acidic by addition of 2ml nitric acid (2:1). After which 25 ml of the Vanodo-molybdate reagent was added. The solution was diluted to the mark, mixed thoroughly and allowed to stand for 10 minutes. The optical density was measured at 47 mu.

2.2.5 Amino Acids Determination

A modified method of AOAC (2000) was used for amino acid analysis. Sixty milligrams of freeze-dried sample were hydrolyzed with 8 ml of 6 M HCl under vacuum at 110 ± 3 °C for 24 hours. After cooling, the hydrolysate was washed with distilled water, filtered using Whatman No 1:11μm filter paper and dried at 60 ± 3 ºC (also under vacuum) in a rotary evaporator. The dried sample was then dissolved in 0.01M HCl. The amino acids in the hydrolysate were separated and quantified by injecting 50 μl into a Hitachi 835-50 amino acid analyzer equipped with a 2.6 mm ×150 mm ion exchange column coated with resin 2619#. The column temperature was 53 °C. Sodium citrate buffers (pH 3.3, 4.3, and 6.3) were used as eluents with a flow rate of 0.225 mL min^{-1}. The absorbance of the amino acids was detected by a 166 Detector (Beckman Instruments, California United States) at 570 nm and the amino acids were quantified by calibration curves using standard concentration.

2.3 Determination of Anti-Nutritional Factors

2.3.1 Phytochemical/Antinutrient Determinations

Oxalate content determination: Oxalate content in the food samples was determined using methods of AOAC (2005). One gram of the sample was weighed into a conical flask. 75 mL of 3M H_2SO_4 was added, and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 hour and then filtered using Whatman No 1: 11 μm filter paper. 25 cm^3 of sample filtrated were titrated against a 0.1 N $KMnO_4$ solution to the final point (pink colour) that persisted for at least 30 sec. The oxalate content of each sample was calculated.

Phytate content determination: The phytate content of each sample was determined using the method described

by Latta and Eskin (1980). Two grams were weighed into 250 ml conical flask. 100 mL of 2% conc. HCl were used to soak the samples then it was filtered using Whatman No 1: 11 μm filter paper. Fifty milliliters (50 mL) of each sample filtrate were added to 100 mL of distilled water in a 250 ml beaker to improve acidity. Ten milliliters (10 mL) of 0.3% ammonium thiocyanate solution was added to each sample solution as indicated and titrated with standard iron chloride solution which contained 0.00195g iron/mL and the end point was signified by brownish – yellow colouration that persisted for 5 min. The percentage of the phytic acid was calculated.

Tannin content determination: Tannin contents were determined by the modified vanillin-HCl methods Latta and Eskin (1980). Two grams sample was extracted with 50 ml 99.9% methanol for 20 minutes at room temperature with constant agitation. After centrifugation for 10 min. at 653 rpm, 5 ml of vanillin-HCl (2% Vanilli and 1% HCl) reagent were added to 1 ml aliquots, and the colour developed after 20 min. at room temperature was read at 500 nm. Correction for interference light natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction, but without vanillin reagent. A standard curve was prepared using Catechin (Sigma Chemical, St. Louis, MO) after correcting for blank, and tannin concentration was expressed in g/100 g.

Determination of Trypsin inhibitor: The Trypsin activity of the samples was determined using the method of Prokopet and Unlenbruck (2002). The inhibitor extract was prepared, the powder samples were defatted with petroleum ether and methanol. One gram of each sample was dispersed in 50 mL of 0.5 M NaCl solution. The mixture was stirred for 30 minutes at room temperature and centrifuged at 1500 rpm for 5 min. The supernatants were filtered and the filtrates used for the assay. Two (2) mL of the standard Trypsin solution was added to 10 mL of the substrate of each sample. The absorbance of the mixture was taken at 410 nm using 10 mL of the same substrate as blank.

2.4 Functional Properties Determinations

Water Absorption Capacity (WAC): Water Absorption Capacity (WAC) was determined using the method of Adebowale et al., 2005. 10 ml of distilled water was added to 1 g of the sample in a beaker. The suspension was stirred using magnetic stirrer for 3 minutes. The suspension obtained was thereafter centrifuged at 3500 rpm for 30 minutes, and the supernatant was measured into a 10 ml graduated cylinder. The water absorbed by the flour was calculated as the difference between the initial volume of the sample and the volume of the supernatant.

$$\text{WAC (\%)} = \frac{Weight\ of\ water\ absorbed \times density\ of\ water \times 100}{weight\ of\ sample}$$

Determination of Least gelation concentration: Gelation property was determined using the method described by Adebowale et al. (2005). Appropriate sample suspensions of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 g were weighed into 10 mL distilled water each to make 20% (w/v) suspension. The test tubes containing these suspensions were heated in a boiling water bath for 1 hour, followed by rapid cooling under running tap water. The test tubes were then cooled for an hour. The least gelation concentration was determined as the concentration when the sample from the inverted test tubes did not slip or fall. The analysis was carried out in triplicate.

$$\text{Least gelation (\%)} = \frac{weight\ of\ sample}{10ml\ of\ water} \times 100$$

Swelling capacity determination: This was determined by the method described by Ikegwu et al. (2010) with modification for small samples. One gram of the flour sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80 ºC for 30 min under continued shaking. After heating, the suspension was centrifuged at $1000 \times g$ for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as follows: swelling power = weight of the paste / weight of dry flour

Bulk density determination: The procedure of Okaka and Potter (1979) was used to determine the bulk density. A 100 mL graduated cylinder was weighed and recorded as W_1, 15 g of sample were put into the cylinder, tapped hermitically to eliminate air space between the flour, the volume was noted and new mass was recorded as W_2. The bulk density was computed as follows:

$$\text{Bulk Density (BD)} = \frac{Mass\ of\ the\ sample}{Volume\ of\ the\ cylinder}$$

2.5 Nutritional Evaluation of Samples

Forty-two weaning albino rats aged 28-35 days with average initial weight of 33-60 g were obtained from the

Central Animal House, College of Medicine University of Ibadan, Nigeria. The rats were randomly distributed into six groups comprising seven rats each. The rats were housed in individual metabolic cages in a room at 25 ± 2 °C with facilities for urine and fecal collection. The rats were acclimatized for four days and thereafter fed with three experimental diets, basal and control (*Ogi*- a local complementary food and *Cerelac*- a commercial formula) diets with water *ad libitum* daily for 28 days. The feed intake was measured daily and body weight and length at 3 days intervals. The fecal droppings of the rats were collected daily, dried at 85 ± 3.0 °C to a constant weight and then ground into powder for fecal nitrogen determination. Urine samples were collected in sample bottles containing 0.1 N HCl to prevent loss of ammonia and stored in a freezer until analyzed for urinary nitrogen. Data on feed consumption and spilled food were collected by recording the feed measured out for each rat at the beginning and the quantity remaining after feeding. Gain or loss in weights of the rats was also recorded. Feacal and urinary nitrogen of the rats were determined by Kjeldhal method (AOAC, 2005). Protein Efficiency Ratio (PER), Net Protein Utilization (NPU), Biological Value (BV), True Digestibility (TD), Net Protein Retention (NPR) were calculated (FAO/WHO, 1989; AOAC, 2000). The experimental rats were sacrificed with chloroform at the end of 28 days, dissected and blood was collected through cardiac punctured into bijour bottles containing ethylenediaminetetraacetic acid (EDTA). The bottles were immediately capped and the content rocked gently. The blood was used for subsequent hematological studies.

2.6 Determination of Hematological Indices

Determination of packed cell volume (PVC): The blood samples were mixed well but gently for 2 minutes, drawn up a 75 x 1.5 mm capillary tube for $^3/_4$ of the length, one end of the capillary tube was sealed with sealant and then placed in a haematocrit centrifuge ensuring that the sealant is at the outer end before closing the centrifuge lid, the tube was centrifuged at 12,000 rpm for 4 minutes, the tube then placed in a reader and the reading recorded. The reading was expressed as percentage of packed red cells to total volume of the whole blood.

Determination of hemoglobin (Hb): The blood samples were mixed gently for one minute, drawn into 0.2mL pipette to the mark, and then expelled into 4 mL of Drabklin's solution. The pipette was washed thoroughly, re-filled with blood and then expelled into the Drabkin's solution. The tube was stopped and mixed and allowed to stand for 5 min until a full color was developed. Standard was prepared as above using a blood sample of known hemoglobin concentration. A green (624 nm) filter was used, setting the colorimeter to zero using the Drabkin solution as blank. The sample and standard blood dilutions on the colorimeter were read

$$Hb = \frac{\text{Absorbance of standard X Standard concentration}}{\text{Absorbance of sample}} (g/dl)$$

Determination of red blood cells count (RBC): The blood samples were mixed thoroughly by repeated inversion; 0.2 ml pipette was used to draw the blood up to the mark before expelling it to a 4 ml of diluting fluid in a bijou bottle and then the pipette washed thoroughly by alternately drawing up and expelling the dilute fluid. The diluted blood were mixed for at least half minute by inversion and a fine Pasteur was used to fill the counting chamber

Determination of white blood cells count (WBC): The white blood cell count samples were diluted in the same way as for the red blood cells but using a 0.05 ml blood pipette and 0.95 ml of diluting fluid.

2.7 Statistical Analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) software version 16.0. The mean and standard deviations of the analyses were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means using Duncan Multiple Range Test at $P < 0.05$.

3. Results and Discussion

3.1 Proximate and Mineral Composition of Popcorn-Moringa Based Complementary Foods

The proximate and mineral composition of blanched and fermented popcorn-*moringa oleifera* flour blends and control samples are presented in Table 1. The moisture content of blanched popcorn-moringa leaves (BPM) formulation (6.73 ± 0.33 g/100 g) was lower than that of fermented popcorn-moringa leaves flour (FPM) formulation (8.02 ± 0.24 g/100 g) and lower when compared with Ogi (8.31 ± 0.57 g/100 g) and Cerelac (11.3 ± 0.50 g/100 g), respectively. This observation indicates that BPM and FPM samples may have longer shelf life, than 'Ogi' and Cerelac because of their lower moisture contents. Studies have shown that moisture content in food products facilitate the growth of microorganisms, which in turns causes spoilage and low nutritional qualities of the food products (Udensi et al., 2012; Oyarekua, 2013). Protein content of FPM (21.27 ± 0.20 g/100

g) had higher values than that of BPM (15.99 ± 0.14 g/100 g) and control samples, which include *Cerelac* (15.75 ± 0.01 g/100 g) and 'Ogi' (6.52 ± 0.31 g/100 g) respectively. However, the protein contents of popcorn-moringa leaves combinations in this present study were higher than FAO/WHO (1991) recommended value for infant complementary food (≥ 15 g/100 g), and also higher than the value reported for complementary foods formulated from sorghum, sesame, carrot and crayfish (Onabanjo et al., 2009). Energy value of FPM blend (393.94 ± 0.39kcal.) was also higher than that of BPM (389.69 ± 1.40 Kcal) sample, however, energy values of formulated diets were lower when compared with those of control samples, that is, 'Ogi' (418.08 ± 0.47 kcal) and *Cerelac* (431.58 ± 0.01kcal). This observation could be attributed to low carbohydrate contents that were observed in popcorn and moringa leaves flour blends. Nutritionally, The high protein and energy values observed in this study, particularly FPM blend, showed that the formulations are suitable for infants complementary food; and also could be substituted for traditional complementary foods, that is, cereal gruel, which had been implicated as one of the major causes of protein–energy malnutrition among weaning aged children in Nigeria and other developing countries (Anigo et al., 2009). It has been proven that most families in developing countries, including Nigeria, are unable to feed their children with the high cost fortified, nutritious, proprietary complementary foods, due to poverty (Mosha et al., 2000; Amankwah et al., 2009; Bruyeron et al., 2010; Muhimbula et al., 2011). Hence, such families are always depending on low cost family diets or mainly of un-supplemented cereal porridges made from maize, sorghum and millet, which are low in protein and energy density (Eka et al., 2010). To improve nutritional quality of complementary foods, studies have advocated for the use of cereal and legumes or other locally available food materials in combinations (ACC/SCN, 2001; Ibeanu, 2009), which help to increase the protein and energy density of complementary foods fed to young children in developing countries. FAO/WHO (1985) has recommended that foods fed to infants should be adequate in protein and energy dense, because low energy dense foods tend to reduce total energy intake and other essential nutrients in children. Evidence has shown that high-energy foods are necessary for children to cover their energy needs considering the small size of their stomach (Solomon, 2005).

Mineral contents of BPM were higher in zinc, iron, potassium, sodium and phosphorous, while FPM sample was higher in copper, calcium and magnesium. The mineral contents in the formulated diets were comparatively higher than Ogi, but lower than that in *Cerelac*. The lower mineral content observed in the formulated diets when compared with the *Cerelac* could be attributed to the facts that the *Cerelac*, a commercial complementary food, was fortified with micronutrients during its production and such was not applied to the formulated diets in this present study. To compensate for lost of macronutrients and micronutrients in processed foods, a number of researchers have advocated for food fortification, particularly infants foods, during the production process (Rosalind et al., 2000; Lutter & Dewey, 2003). The Ca/P and Na/K molar ratios of popcorn-moringa leaves flour blends ranged from 0.55 to 1.17 and 0.51 to 0.71, respectively. These observations showed that all the samples met the recommended values for Na/K (<1.0) and Ca/P (>2.0), except BPM for Ca/P, which indicate that the formulated diets would support bone and teeth formation in children and also would not pose any danger to heart of the infant whenever taken as complementary food.

Table 1. Proximate composition (g/100g) and mineral composition (mg/100g) of complementary foods formulated from popcorn and *Moringa oleifera* leaf flour

Nutrient	FPC	BPM	FPM	Ogi	Cerelac	Recommended values
Macronutrient composition						
Moisture	5.43 ±0.47[c]	6.73 ±0.33 [b]	8.02 ±0.24 [b]	8.31 ±0.57[b]	11.3 ±0.50[a]	<5
Ash	0.87[b] ±0.07	4.33 ±0.30[a]	3.87 ±0.03[a]	1.09 ±0.01[b]	3.16 ±0.01[a]	<3
Protein	14.37 ±0.52[c]	15.99 ±0.14[b]	21.27 ±0.20[a]	6.52 ±0.31[d]	15.75 ±0.01[bc]	>15
Fat	5.85 ±1.63[c]	8.67 ±0.14[b]	10.51 ±0.11[a]	5.17 ±0.11[c]	10.53 ±0.02[a]	10-25
Fiber	0.81 ±0.21[b]	2.35 ±0.04[a]	2.76 ±0.02 [a]	0.85 ±0.01[b]	2.11 ±0.01[a]	<5
Carbohydrate	78.09 ±1.25[b]	61.91 ±0.74[d]	53.55 ±0.47[e]	86.38 ±0.21[a]	68.42 ±0.01[c]	64
Energy	422.53 ±8.91[a]	389.69 ±1.40[b]	393.94 ±0.39[b]	418.08 ±0.47 [a]	431.58 ±0.01[a]	400-425
Minerals composition						
Zinc	2.84 ±0.32[b]	0.16 ±0.01[c]	0.12 ±0.01[cd]	0.08 ±0.00[d]	5.00 ±0.00[a]	3.2
Iron	4.12[b] ±0.04	1.83 ±0.01[c]	1.81 ±0.01[c]	0.26 ±0.01[d]	7.50 ±0.01[a]	16
Potassium	122.59 ±1.68[c]	270.64 ±0.25[b]	122.86 ±0.05[c]	102.39 ±1.01[d]	635.00 ±0.00[a]	516
Sodium	140.71 ±0.45[b]	137.61 ±0.05[c]	87.62 ±0.07[d]	14.56 ±0.04[e]	145.00 ±0.00[a]	296
Calcium	134.80[b] ±0.07	45.87 ±0.02[e]	85.71 ±0.02[c]	68.66 ±0.35[d]	600.00 ±0.01[a]	500
Magnesium	31.44[d] ±0,96	284.40 ±0.02[b]	285.71 ±0.01[a]	34.91 ±0.01[c]	0.00[e]	76
Phosphorous	142.51[b] ±14.62	84.12 ±0.02[c]	73.28 ±0.01[d]	85.95 ±0.02[c]	400.00 ±0.01[a]	456
Lead		-	-	-	-	-
Na/K	1.15 ±0.02[a]	0.51 ±0.06[c]	0.71 ±0.01[b]	0.14 ±0.01[e]	0.23 ±0.02[d]	<1
Ca/P	0.95 ±0.01[c]	0.55 ±0.0[e]	1.17 ±0.01[b]	0.80 ±0.01[d]	1.50 ±0.01[a]	1.6-3.6

Mean values with the same superscript in a row are not significantly different (P>0.05), * RV- Recommended values for infant complementary foods (*FAO/WHO, 1991*).

Key: FPM (Fermented Popcorn-Moringa leave flour), BPM (Blanched Popcorn-Moringa leave flour).

3.2 Amino Acids Profile of Popcorn-Moringa Based Complementary Foods

The amino acids profile of the formulated complementary food samples are shown in Table 2. The predominant amino acid in the blends was glutamic acid and the finding agreed with other researchers, who reported that glutamic and aspartic acids are usually the most abundant amino acids in plant based food products (Adeyeye, 2004; Aremu et al., 2006); while methionine was the least concentration. Arginine and histidine which are essential amino acids for infants' growth and development were higher in fermented formulation (FPM) than blanched formulation (BPM). However, these amino acids (arginine and histidine) were lower than the reference egg protein (6.3 g/100 g) and RDA value of (6.6 g/100 g) (FAO/WHO/UNU, 1985). Total essential amino acids in FPM blend (25.99 g/100 g protein) was higher than that of BPM blend (24.56 g/100 g protein), and both were

higher than 'Ogi' (18.32 g/100 g protein), but lower when compared with that of *Cerelac* (31.73 g/100 g protein). The concentration of total essential amino acids observed in FPM blend compared with BPM blend is likely due to the activities of microorganisms, which involves utilizing other nutrients like carbohydrate in the food product to synthesize amino acids which the organisms need for their growth (Cronk et al., 1977).

Table 2. Amino acids (g/100g crude protein) profile of blanched and fermented popcorn-*Moringa oleifera* leaves blends and control samples

Amino acids	FPC	BPM	FPM	Ogi	Cerelac	*RDA
Non essential amino acids (TNEAA)						
Alanine	5.85 ±0.05[a]	3.09 ±0.01[d]	3.47 ±0.01[c]	3.42 ±0.19[c]	4.42 ±0.02[b]	
Aspartic acid	7.21 ±0.02[b]	6.17 ±0.02[d]	6.88 ±0.01[c]	6.12 ±0.02[e]	9.26 ±0.01[a]	
Serine	0.26 ±0.02[e]	4.02 ±0.02[d]	4.22 ±0.02[c]	4.78 ±0.01[a]	4.33 ±0.02[b]	
Glutamic acid	7.59 ±0.01[e]	14.72 ±0.02[d]	15.14 ±0.01[c]	17.64 ±0.01[b]	19.54 ±0.01[a]	
Proline	0.55 ±0.01[e]	2.72 ±0.02[b]	2.68 ±0.01[b]	2.49 ±0.01[c]	3.26 ±0.02[a]	
Glycine	0.36 ±0.01[e]	4.79 ±0.01[c]	5.16 ±0.01[b]	4.15 ±0.02[d]	5.88 ±0.01[a]	
∑NEAA	21.82 ±0.02[e]	35.52 ±0.07[d]	37.56 ±0.04[c]	38.63 ±0.14[b]	46.73 ±0.04[a]	
Essential amino acids (TEAA) for infant						
Lysine	2.18 ±0.02[c]	3.54 ±0.02[b]	3.65 ±0.02[b]	1.71 ±0.01[d]	4.13 ±0.01[a]	5.8
Arginine	4.06 ±0.02[c]	1.72 ±0.01[e]	1.89 ±0.02[d]	4.84 ±0.02[b]	9.12 ±0.02[a]	2
Threonine	1.23 ± 0.01[d]	4.11 ±0.03[b]	4.38 ±0.01[a]	1.09 ±0.01[d]	3.80 ±0.10[c]	3.4
Valine	1.38 ±0.01[e]	3.03 ±0.02[c]	3.36 ±0.01[b]	2.69 ±0.01[d]	4.79 ±0.01[a]	3.5
TSAA (Meth.+ cystein)	1.10 ±0.00[e]	3.12 ±0.01[a]	3.02 ±0.02[c]	3.08 ±0.01[b]	2.79 ±0.01[d]	2.5
Isoleucine	2.27 ±0.01[d]	2.33 ±0.01[c]	2.34 ±0.02[c]	3.56 ±0.01[b]	4.23 ±0.01[a]	2.8
Leucine	3.78 ±0.04[c]	4.96 ±0.02[b]	5.23 ±0.02[a]	3.75 ±0.01[c]	5.25 ±0.01[a]	6.6
TArAA(Phenyl.+Tyro)	3.27 ±0.01[c]	6.58 ±0.02 [ab]	6.48 ±0.01[ab]	6.08 ±0.02 [b]	8.23 ±1.25[a]	6.3
Histidine	0.55 ±0.05[e]	1.71 ±0.01[c]	1.89 ±0.01[b]	1.57 ±0.01[d]	2.04 ±0.02[a]	1.9
Tryptophan	ND	ND	ND	ND	ND	1.1
∑EAA	19.82 ±0.03 [d]	31.13 ±0.08[b]	32.36 ±0.23[b]	28.38 ±0.06[c]	44.51 ±1.38[a]	
∑AA	41.64 ±0.11[d]	66.65 ±0.14[c]	69.92 ±0.26[b]	67.01 ±0.09[c]	91.23 ±1.42[a]	

Mean values with the same superscript in a row are not significantly different (P >0.05),

*FAO/WHO (1991), FPC (fermented popcorn), FPM (Fermented Popcorn-Moringa leave flour), BPM (Blanched Popcorn-Moringa leave flour).

3.3 Anti-Nutrient Factors in Popcorn- Moringa oleifera Leaves Formulations

The concentrations of anti–nutrients in the formulated complementary food samples are shown in Table 3. The values of oxalate, phytate and trypsin inhibitor in fermented popcorn-moringa leaves blend (FPM) were lower when compared with the blanched popcorn-*Moringa oleifera* leaves blend (BPM). It was generally observed in this study that the antinutrient compositions of the formulations were generally low and they are within the tolerable levels. For instance, the oxalate and tannin contents of the formulated complementary foods were lower compared with the complementary food based on Soybean and Tigernut (Ikpeme-Emmanuel et al., 2012). Study has shown that oxalates in large amounts bind with calcium forming calcium oxalate, which is insoluble and not absorbed by the body (Ladeji et al., 2004). Oxalates are considered poisonous at high concentration, but harmless when present in small amounts (Chai & Liebman, 2004). High oxalate level in food has been implicated as the cause of kidney stones because high level of oxalates increases calcium absorption in the kidney (Chai & Liebman, 2004). Tannin has been implicated to form insoluble complexes with proteins thereby reducing digestibility and utilization of food proteins, interference with the absorption of Iron and inhibition of trypsin, chymotrypsin, amylase and lipase (Griffiths & Mosley, 1980; Delumen & Salamat, 1980). Studies have reported that combination of blanching or cooking and fermentation improved the nutritional quality of food products and also reduced the anti-nutritional factors in the food product to a safe level (Paredes-López & Harry, 1988; Obizoba & Atii, 1991).

Table 3. Anti-nutritional content (mg/100g) of complementary foods formulated from *Moringa oleifera* leaves and popcorn

Antinutrients	BPM	FPM
Oxalate	6.26±0.045	4.95±0.09
Phytate	23.07±0.001	20.19±0.41
Tannin	0.06±0.005	0.09±0.00
Trypsin inhibitor	33.25±0.255	29.79±0.13

Key: FPM (Fermented Popcorn-Moringa leave flour), BPM (Blanched Popcorn-Moringa leave flour).

3.4 Functional Properties of Popcorn-Moringa oleifera Leave Formulations

Functional properties of the complementary foods formulated from popcorn and *Moringa oleifera* leaves are presented in Table 4. Bulk density of BPM and FPM were 0.73 g/cm^3 and 0.70 g/cm^3, respectively. The BPM had higher values in water absorption capacity (68.5 ml/g) and least gellation (0.63%) when compared with FPM blends (55.5 ml/g and 0.62%, respectively), while FPM had higher value in swelling capacity (1.25%) than BPM (1.05%). Comparatively, it was observed that the BPM and FPM blends were higher in bulk density and water absorption capacity than in *Cerelac*, but lower in swelling capacity and least gellation. The higher values of water absorption capacity and bulk density that were observed in this study compared with the control samples do not limit the nutritional advantages of these products. However, these values were similar to other plant-based food products (Masood & Rizwana, 2010; Gernah et al., 2012; Oyarekua, 2013). Higher water absorption capacity indicates higher protein content in the formulations, which absorbs and binds with more water (Otegbayo et al., 2000). Scientific finding has shown that high water absorption capacity indicates that food samples hold large volume of water during cooking into gruels, to yield voluminous low energy and nutrient food (Omueti et al., 2009). According to WHO (2003), appropriate complementary food is the one which produce a gruel or porridge that is neither too thick for the infant to consume nor so thin that energy and nutrient density are reduced. Therefore, low water absorption capacity is desirable in complementary food for making thinner gruels with high caloric density per unit volume. The Bulk Density (BD) is a reflection of the load the sample can carry if allowed to rest directly on one another. The lower the bulk density value, the higher the amount of flour particles that can stay together thereby increases the energy content derivable from such diets (Onimawo & Egbekun, 1998). Evidence has shown that high bulk density limits the caloric and nutrient intake per feed of a child, because of the small capacity of the child's stomach that would not be able to accommodate large volume of food to satisfy their energy and nutrient requirements (Omueti et al., 2009). Besides, bulk density is also important in the packaging requirement and material handling of the complimentary diet (Karuna et al., 1996).

Table 4. Functional properties of complementary foods formulated from *Moringa oleifera* leaves and popcorn

Parameters	Bulk Density (g/cm³)	Water absorption capacity (ml/g)	Swelling Capacity(%)	Least Gellation (%)
BPM	0.73±0.00	6.9±0.05	1.05±0.05	0.63±0.03
FMP	0.70±0.01	5.6±0.05	1.25±0.05	0.62±0.03
Ogi	0.66±0.01	1.82±0.02	0.90±0.03	9.00±1.11
Cerelac	0.56±0.03	2.31±0.21	2.43±0.03	14.00±1.21

FPM (Fermented Popcorn-Moringa leave flour), BPM (Blanched Popcorn-Moringa leave flour)

3.5 Protein Quality and Heamatological Evaluations

The protein digestibility indices of the formulated diets and control samples are presented in Figure 1. The nutritional indices for evaluating protein digestibility of the formulated diets in rats ranged as follows: 76-88% for food efficiency (FE), 95.9-96.7% biological value (BV), 79.5-84.2% net protein utilization (NPU) and 2.69-4.32 protein efficiency ratio (PER). Comparatively, the popcorn-*Moringa oleifera* leaves sample had higher values in FE, BV, PER than blanched popcorn-*Moringa oleifera* leaves sample and Ogi, but lower when compared with the *Cerelac*. The BV and PER of the formulations met the FAO/WHO (1989) recommended values of 70% and 2.7, respectively. These indicate that the protein content in the formulations were of good qualities and are suitable to support growth and development in infant.
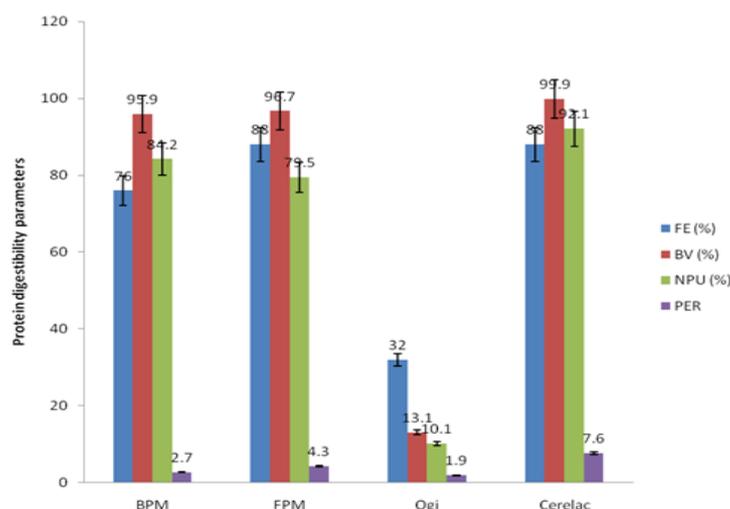
Figure 1. Protein digestibility of formulated diets and weight of organs of Albino rats fed with the diets (p < 0.05)

Key: FPM (Fermented Popcorn-Moringa leave flour), BPM (Blanched Popcorn-Moringa leave flour), FE (Food efficiency), BV (Biological value), NPU (Net protein utilization), PER (Protein efficiency ratio)

Growth rates of albino rats fed with the formulated diets and control samples are shown in Figures 2-4. The albino rats fed with the FPM sample had higher growth rate when compared with those rats fed with BPM sample and Ogi, but lower than those rats fed with *Cerelac*. The higher growth rated recorded for those animals fed with the *Cerelac* compared with those fed with formulated diets could be attributed to its food composition, i.e., milk-based and large quantity of food intakes by the rats in *Cerelac* group due to the attractive flavor and taste of the product. This observation agreed with the reports of Sodipo and Fashakin (2011) and Ijarotimi (2006), who reported higher weight gained in animals fed with *Cerelac* and Nutrend, respectively. The weight of the organs, i.e., kidney, liver and heart, of albino rats fed with BPM and FPM diets were well developed than those rats fed with 'Ogi', but comparable with those in *Cerelac* group (Figure 5).
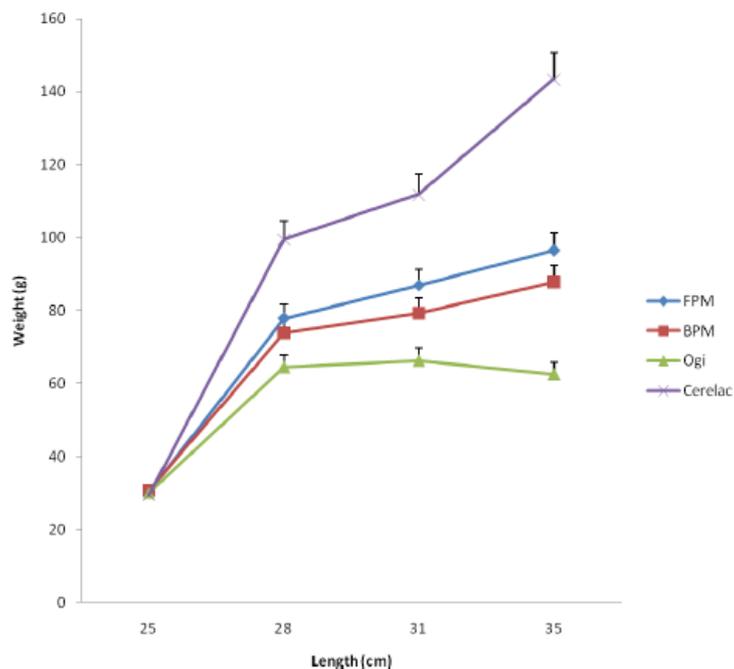
Figure 2. Nutritional status of the Albino rats fed with formulated diets and control food samples (Cerelac and ogi) using weight-for-length nutritional index
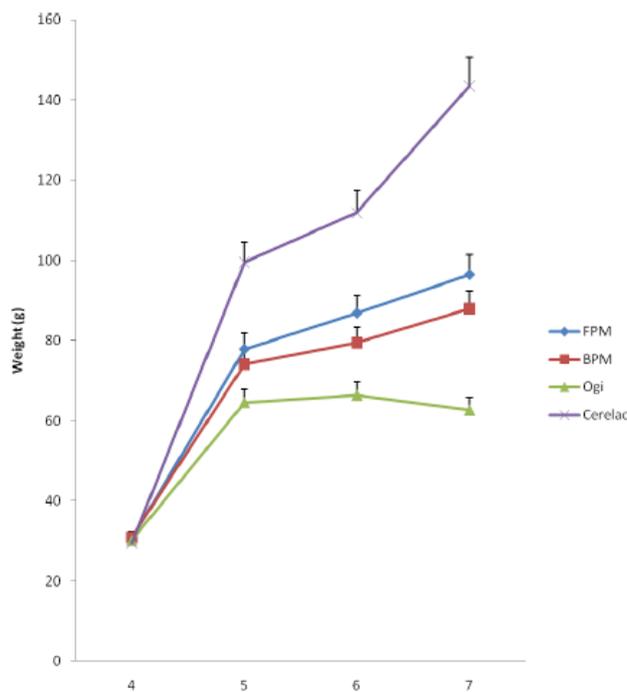
Figure 3. Nutritional status of the Albino rats fed with formulated diets and control food samples (Cerelac and ogi) using weight-for-age nutritional index
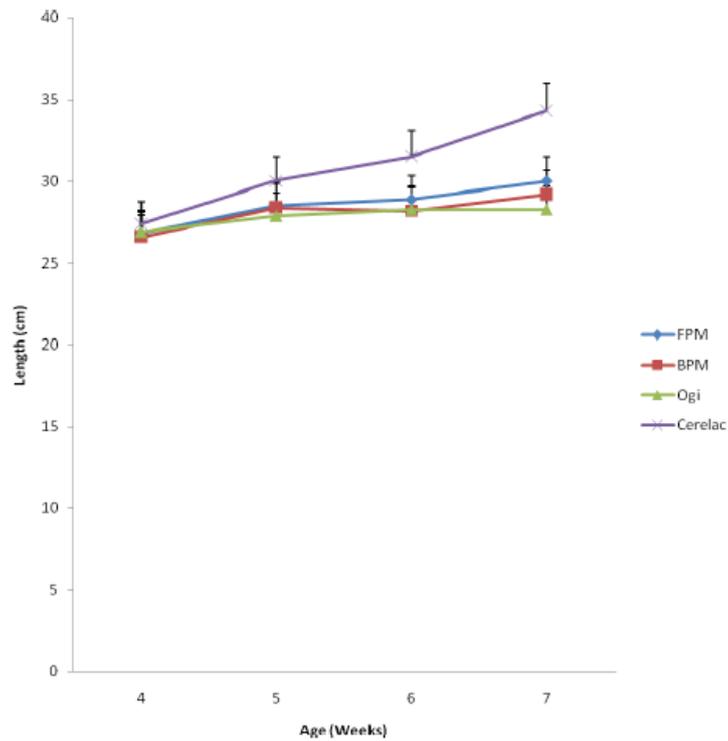
Figure 4. Nutritional status of the Albino rats fed with the formulated diets and control food samples (Cerelac and ogi) using length-for-age nutritional index
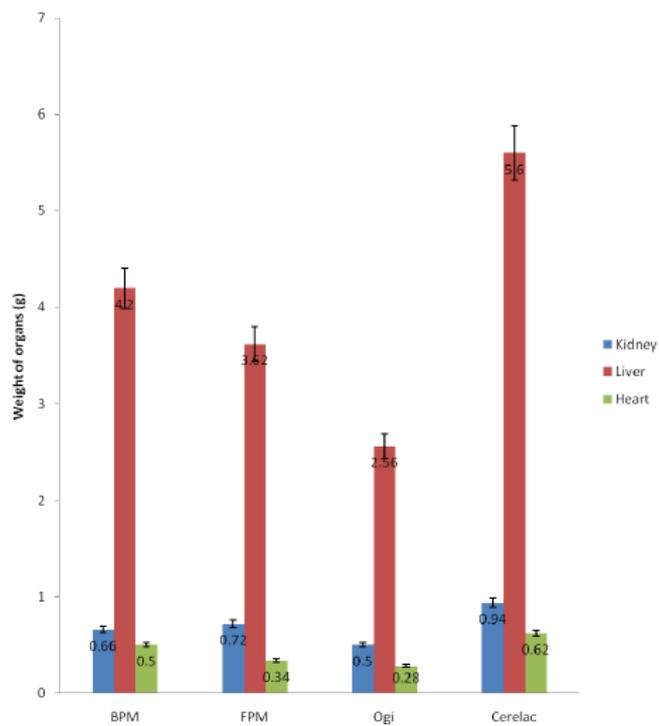
Figure 5. Weight of organs of albino rats fed with the formulated diets and control food samples (*Cerelac* and ogi) (p<0.05)

Key: FPM (Fermented Popcorn-Moringa leave flour), BPM (Blanched Popcorn-Moringa leave flour).

The heamatological properties of Albino rats fed with the formulated diets and control samples are presented in Table 5. The pack cell volume (PCV), red blood cells and white blood cells of Albino rats fed with the BPM diet were higher than FPM, and both diets were higher than those rats fed with control samples, except in *Cerelac* group. The growth rate and non-atrophy of livers, kidneys and hearts of rats fed with the formulated diets couple with heamatological values further showed that the popcorn-moringa leaves based diets are good substitutes for 'Ogi' or other family diets used as complementary, which have been implicated as one of the causes of protein-energy malnutrition among weaning aged children in developing countries.

Table 5. Haematological Properties of popcorn-based infant food supplemented with *Moringa oleiera* leaves flour

Heamatological indices	Ogi	*Cerelac*	FPM	BPM	Baseline values
ESR (mm)	1.0[a]	1.0[a]	1.0[a]	1.0[a]	1.0[a]
PCV (%)	37.0[ab]	41.0[a]	38.0[ab]	40.0[a]	36.0[b]
RBC ($x10^6 mm^3$)	493[d]	651[a]	504[c]	572[b]	488[e]
WBC ($x10^3 mm^3$)	221[b]	186[d]	234[a]	208[c]	213[c]
Hemoglobin (g/100ml)	12.3[a]	13.6[a]	12.6[a]	13.3[a]	12.0[a]
Lymphocytes (%)	60.0[a]	62.0[a]	60.0[a]	64.0[a]	65.0[a]
Neutrophils (%)	31.0[a]	28.0[b]	32.0[a]	26.0[b]	26.0[b]
Monocytes (%)	5.5[a]	6.0[a]	5.0[a]	6.0[a]	5.0[a]
Eosinophils (%)	2.0[a]	3.0[a]	2.0[a]	3.0[a]	4.0[a]
Basophils (%)	1.0[a]	1.0[a]	1.0[a]	1.0[a]	0.0[b]

Mean values with the same superscript in a row are not significantly different (P>0.05)

Key: FPM (Fermented Popcorn-Moringa leave flour), BPM (Blanched Popcorn-Moringa leave flour).

4. Conclusion

The present study established the chemical compositions and nutritional qualities of infant food formulated from popcorn and *Moringa oleifera* leaves flour combinations. The findings showed that the formulated diets, particularly fermented popcorn-moringa (FPM) blend, were better in terms of protein contents, essential amino acids and ability to support growth and development in experimental animals than local complementary food (Ogi). Hence, these formulations could be used as infant food, particularly for underprivileged children, who cannot have access to qualitative complementary foods.

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