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Section: Food Quality and Functionality

Determination of Nutrient Composition and Protein Quality of Potential Complementary Foods Formulated from the Combination of Fermented Popcorn, African Locust and Bambara Groundnut Seed Flour

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The aims of this study are to produce and evaluate nutritional quality of complementary foods using popcorn, bambara groundnut and African locust beans.

The food materials were fermented, oven dried, milled and sieved into flour. The flours were mixed as follows: fermented popcorn-African locust bean (FPA) (70% popcorn, 30% African locust bean), fermented popcorn-bambara groundnut (FPB) (70% popcorn, 30% bambara groundnut) and fermented popcorn-African locust bean-bambara groundnut (FPAB) (70% popcorn, 20% bambara groundnut, 10% African locust bean). Physicochemical, sensory and nutritional properties of food samples were determined using standard methods.

Protein content of FPAB (26.87 ± 1.07 g/100 g) was significantly higher than FPB (20.87 ± 1.02 g/100 g) and FPA (20.49 g/100 g) respectively. For mineral composition, potassium had the highest value in FPA (173.75 ± 0.21 mg/100 g), FPB (157.45 ± 0.25 mg/100 g) and FPAB (132.75 ± 0.15 mg/100 g), while copper was the least mineral. The percentage recommended daily allowance (RDA) met by total essential amino acid (TEAA) ranged between 55.99% for FPA and 82.33% for FPB. The predicted protein efficiency ratio (P-PER) range between 0.54 and 1.43 for FPB and FPAB respectively; while biological value (BV) was between 20.13 and 39.94%. The oxalate, tannin, phytate and trypsin inhibition of popcorn-based food samples was reduced to a minimum level.

It could be concluded that FPAB had a better nutritional quality over FPA and FPB using selected nutritional indices; hence, FPAB blends could be used as complementary food for infants. However, nutritional and biochemical studies of the formulations are needed to further substantiate their nutritional potentials.

INTRODUCTION

Scientific study has proved that breast milk is the perfect food for the infant during the first six months of life. It contains all the nutrients and immunological factors an infant requires to maintain optimal health and growth [UNICEF, 1999]. However, at the age of six months and above when the child is undergoing rapid growth, physiological maturation and development, breast milk is no longer sufficient to meet the nutritional needs of the infant. Nutritious complementary foods are therefore introduced – also known as weaning foods – which typically cover the period from six to twenty four months of age in most developing countries [WHO/OMS, 2000]. Poor nutrition during this critical period of life may increase the risk of growth faltering and micronutrient deficiencies, and may have adverse effects on health and mental development. Hence, improved complementary foods at this period of life are essential for the child normal growth and cognitive development.

In Nigeria, the introduction of complementary food usually starts between age 4 and 6 years; and usually involves the use of a semi-liquid porridge prepared locally by the mother from staple cereals or tubers [Bentley *et al*., 1991; Nout, 1993; Guptill *et al*., 1993]. Complementary feeding period is the time when malnutrition starts in many infants, contributing significantly to the high prevalence of malnutrition in children less than 5 years of age worldwide [Daelmans & Saadeh, 2003]. The complementary food is often associated with low energy-density and other essential nutrients; and in many parts of developing countries it is usually given too earlier or late in insufficient amounts to meet the nutritional needs of infant [Onyango, 2003; Muhimbula *et al.*, 2011]. Scientific research has reported that timely introduction of appropriate complementary foods promotes good nutritional status and growth in infants and young children [Michaelsen *et al*., 2000; Domellof *et al*., 2002]; and that too early (<6 months) or too late (>6 months) introduction of complementary foods increases infant morbidity [Bhandari *et al*., 2002; Kalanda *et al*., 2006] and mortality [Edmond *et al*., 2006] in many parts of developing countries [Onyango, 2003; Hussein, 2005; NBS and ORC Macro, 2005].

The nutritional adequacy of complementary foods is essential for the prevention of infant morbidity and mortality,

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including malnutrition and overweight. In Nigeria, traditional complementary foods are usually produced from staple cereals and legumes prepared either individually or as composite gruels [Walker, 1990]. Cereal grains are considered to be one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for people in developing countries. However, the nutritional quality of cereals and sensorial properties of their products are sometimes inferior or poor in comparison with milk and milk products. This is because cereal is deficient in certain essential amino acids (*i.e.*, lysine and tryptophan), and additionally is characterized by low starch availability, presence of anti-nutrients (phytic acid, tannins and polyphenols) and the coarse nature of the grains [Vasal, 2001]. Combination of common cereals, which are deficient in lysine but have sufficient amount of sulphur-containing amino acids, with inexpensive plant protein sources like legumes that are rich in lysine can be used to improve the nutritive value of a food product.

FAO/WHO/UNICEF [1971] emphasised the use of local foods formulated at home and guided by the following principles: (i) high nutritional value to supplement breastfeeding, (ii) acceptability, (iii) low price, and (iv) use of local food materials [Dewey & Brown, 2003; Pelto *et al.*, 2003]. Therefore, the aims of the present study are to evaluate the effect of fermentation on nutrient composition, sensory attributes and functional properties and protein quality using predicted protein efficiency ratio (PER), biological value (BV), essential amino acid index and nutritional index (NI) of complementary foods formulated from fermented popcorn, African locust bean and bambara groundnut flour blends; while the second part of the research would involve nutritional and biochemical evaluation of formulated complementary foods using animal model.

MATERIALS AND METHODS

Sources of food materials

The materials, that is, popcorn, bambara groundnut and African locust bean used for the food combinations were obtained from a local market in Akure Township, in March, 2011. The control food samples (Cerelac and ogi) were obtained from a reputable supermarket and vendor, respectively.

Food processing: Fermentation of popcorn, bambara groundnut and African locust beans

Fermented popcorn flour

Fermentation was performed using the microorganisms naturally present on the grain surface. The popcorn seeds were weighed (1 kg), sorted and soaked in hot water and left for 4 days. The grains were washed with distilled water, wet milled with attrition mill (locally fabricated grinding machine), sieved with muslin cloth and allowed to ferment for 3 days. The fermented slurry was decanted, drained, oven dried in a hot air oven at 60ºC (Plus11 Sanyo Gallenkamp PLC, UK) for 20 h, re-milled using a Philips laboratory blender (HR2811 model), sieved using a 60 mm mesh sieve (British Standard) and packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses.

Fermented bambara groundnut flour

The bambara groundnut seeds were weighed (1 kg), sorted to remove foreign substances, washed with distilled water and cooked for one and half hours. The cooked sample was tightly wrapped in plantain leaves for three days to ferment. After, the fermented bambara groundnut seeds were oven dried at 60ºC (Plus11 Sanyo Gallenkamp PLC, UK) for 20 h, milled using a Philips laboratory blender (HR2811 model), sieved using a 60 mm mesh sieve (British Standard) and packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses.

Fermented African locust bean

The African locust beans were weighed (1 kg), washed with distilled water, soaked in warm water for 5 days, dehulled manually and cooked for one and half hours. The cooked sample was tightly wrapped in plantain leaves for one day to ferment. After, the fermented African locust beans were oven dried at 60ºC (Plus11 Sanyo Gallenkamp PLC, UK) for 20 h, milled using a Philips laboratory blender (HR2811 model), sieved using a 60 mm mesh sieve (British Standard) and packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses.

Food formulations

The food samples were formulated with reference to protein requirement of infants (18 g/day) using Nutrisurvey linear programming (2004) software to obtain the following blends: fermented popcorn (70%): Africa locust bean (30%) (FPA); fermented popcorn (70%): bambara groundnut (30%) (FPB); and fermented popcorn (70%): bambara groundnut (20%): African locust bean (10%) (FPAB). Ogi (corn gruel and a traditional complementary food) and Cerelac (a commercial complementary formula) were used as the control food samples.

Macronutrient analysis

Nutrient composition of the food samples was determined using the standard AOAC procedures [2005]. Triplicate samples were used for moisture content in a hot-air circulating oven (Galenkamp). Ash was determined by incineration (550℃) of known weights of the samples in a muffle furnace (Gallenkamp, size 3) (Method No 930.05) [AOAC, 2005]. Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40 to 60℃) in a Soxhlet extractor (Method No 930.09) [AOAC, 2005]. Protein (N × 6.25) was determined by the Kjeldahl method (Method No 978.04) [AOAC, 2005]. Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (Method No 930.10) [AOAC, 2005]. The carbohydrate content was determined by difference. Addition of all the percentages of moisture, fat crude protein, and ash, crude fibre was subtracted from 100%. This gave the amount of nitrogen free extract otherwise known as carbohydrate:

% carbohydrate=100-(%Moisture+%Fat+%Ash+% Crude fibre+%Crude protein)

The sample calorific value was estimated (in kcal/g) by multiplying the percentages of crude protein, crude lipid and car-

bohydrate with the recommended factors (2.44, 8.37 and 3.57, respectively) as proposed by Martin & Coolidge [1978].

Mineral analyses

The method described by AOAC [2005] was used for mineral analysis. The samples were ashed at 550°C. The ash was boiled with 10 mL of 20% hydrochloric acid in a beaker and then filtered into a 100 mL standard flask. This was made up to the mark with deionized water. The minerals were determined from the resulting solution. Sodium (Na) and potassium (K) were determined using the standard flame emission photometer. NaCl and KCl were used as the standards [AOAC 2005]. Phosphorus was determined calorimetrically using the spectronic 20 (Gallenkamp, UK) [Kirk & Sawyer, 1991] with KH_2PO_4 as the standard. Calcium (Ca), magnesium (Mg) and iron (Fe) were determined using Atomic Absorption Spectrophotometer (AAS Model SP9, Cambridge, UK). All values were expressed in mg/100 g.

Amino acid determination

Sample preparation for amino acid analysis

About 2.5 g of each sample were weighed into the extraction thimble and fat was extracted with a chloroform/methanol (2:1, v/v) mixture using a Soxhlet apparatus [AOAC, 2005]. The extraction lasted for 5–6 h.

Hydrolysis of samples

About 30 mg of the defatted sample were weighed into glass ampoules. Seven milliliters of 6 mol/L HCl were added and oxygen expelled by passing nitrogen gas into the samples. The glass ampoules were sealed with a Bunsen flame and put into an oven at 105 ±5°C for 22 h. The ampoule was allowed to cool; the content was filtered to remove humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5 mL of acetate buffer (pH 2.0) and stored in a plastic specimen bottle kept in the deep freezer.

Amino acid analysis

Amino acid analysis was by ion exchange chromatography (IEC) [FAO/WHO, 1991] using the Technicon Sequential Multisample (TSM) Amino Acid Analyser (Technicon Instruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 mL/min at 60°C with reproducibility consistent within ±3%. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two determinations. Norleucine was the internal standard.

Tryptophan

The tryptophan content was determined in a separate analysis. The weighed samples were placed in polypropylene tubes and after the addition of the internal standard (norleucine), they were hydrolysed in 4.67 mol/L KOH containing 1% (w/v) thiodiglycol for 18 h at 110°C. After hydrolysis, KOH was neutralised with 2.4 mol/L perchloric acid, and the supernatant was

adjusted to pH 3.0 with acetic acid. A 20 μL aliquot of the hydrolysed sample was subjected to derivatization as described above. The solution of amino acid standard was supplemented with tryptophan. Quality assurance for the tryptophan determination was obtained by demonstrating that the method yielded the correct number of tryptophan residues for egg white lysozyme. Tryptophan analysis was performed using a Waters C18 reversed phase column (3.9 x150 mm) (Waters Milford, MA) and the solvents and gradient conditions were as described by Hariharan et al. [1993]. Use of this elution protocol was necessary in order to adequately separate tryptophan from ornithine which results from the alkaline hydrolysis of arginine.

Calculated nutritional quality determinations

Nutritional qualities were determined on the basis of the amino acid profiles. The Essential Amino Acid Index (EAAI) was calculated using the method of Labuda et al. [1982] according to the equation below:

$$EAAI = \sqrt[9]{\frac{[\text{Lys } x\text{Threo x Val x Meth x Isoleu x leu x Phynylal x Histi x Trypt}]a}{[\text{Lys } x\text{Threo x Val x Meth x Isoleu x leu x Phynylal x Histi x Trypt}]b}}$$

where: [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and methionine]$_a$ in test sample and [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine]$_b$ content of the same amino acids in standard protein (%) (egg or casein), respectively.

The nutritional index of the food samples was calculated using the formula below:

$$\text{Nutritional index}[\%] = \frac{\text{EAAI x \%protein}}{100}$$

The biological value was calculated according to Oser [1959] cited by Mune-Mune et al. [2011] using the following equation:

BV = 1.09 x Essential amino acid index [EAAI] – 11.7

The Protein Efficiency Ratio [PER] was estimated according to the regression equations developed by Alsmeyer et al. [1974] cited by Mune-Mune et al. [2011] as given below:

PER = -0.468 + 0.454(LEU) – 0.105(TYR)

Anti-nutritional composition of the samples

Phytic acid determination

Phytic acid was extracted from each 3 g of flour samples with 3% trichloroacetic acid by shaking at room temperature followed by high speed centrifugation as described by Wheeler & Ferrel [1971]. This method depends on an iron to phosphorus ratio of 4:6. Five grams of the test sample were extracted with 3% trichloroacetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide.

The precipitate was dissolved in hot 3.2 N HNO and the colour was read immediately at 480 nm[3]. The standard solution was prepared from $Fe(NO_3)_3$ and the iron content was extrapolated from a $Fe(NO_3)_3$ standard curve. The phytate concentration was calculated from the iron results assuming a 4:6 iron:phosphorus molecular ratio. The phytic acid was estimated by multiplying the amount of phytate-phosphorus by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$.

Tannin content determination

Tannin contents were determined by the modified vanillin-HCl methods [Burns 1971; Price *et al*., 1978]. A 2 g sample was extracted with 50 mL of 99.9% methanol for 20 min at room temperature with constant agitation. After centrifugation for 10 min at $653 \times g$, 5 mL of vanillin-HCl (2% vanillin and 1% HCl) reagent was added to 1 mL of 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.

Oxalate content determination

Oxalate was determined by AOAC [2005] method. Briefly, 1 g of the sample was weighed into 100 mL conical flask. Then, 75 mL of 3 mol/L H_2SO_4 were added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whatman No.1 filter paper. The sample filtrate (extract, 25 mL) was collected and titrated against hot (80–90°C) 0.1 N $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30 s. The concentration of oxalate in each sample was obtained from the calculation: 1 mL 0.1 permanganate = 0.006303 g oxalate.

Trypsin inhibition activity determination

The trypsin inhibition activity was assayed in terms of the extent to which an extract of the defatted flour inhibited the action of bovine trypsin [EC 3.4.21.4] on the substrate benzoyl-DL-arginine-p-nitrianilide [BAPNA] hydrochloric [Kakade *et al*., 1974]. The samples (1 g each) were extracted continuously at ambient temperature for 3 h with 50 mL of 10 mmol/L NaOH using a mechanical shaker (GallenKamp orbital shaker Surrey, UK). The pH of the resulting slurry was adjusted to 9.4–9.6 with 1 mol/L NaOH. After extraction, the suspension was shaken and diluted with distilled water so that 1 cm³ of the extract produced trypsin inhibition of 40–60% at 37°C. The respective dilutions were noted. Consequently, TIA was calculated in terms of mg pure trypsin [Sigma type lll, lot 20H0868] from the formula:

$$TIA = \frac{2.632DA \text{ mg pure trypsin inhibited g} - 1 \text{ sample}}{S}$$

where: D is the dilution factor, A is the change in absorbance at 410 mm due to trypsin inhibition per cm³ of diluted sample extract, and S is the weight of the sample.

Functional properties

Water absorption capacity

Water and oil absorption capacities of the flour samples were determined by Beuchat [1977] methods. One gram of the flour was mixed with 10 mL of water or oil in a centrifuge tube and allowed to stand at room temperature (30 ± 2°C) for 1 h. It was then centrifuged at $200 \times g$ for 30 min. The volume of water or oil on the sediment water was measured as well. Water and oil absorption capacities were calculated as mL of water or oil absorbed per gram of flour.

Bulk density

A 50 g flour sample was put into a 100 mL measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density (g/cm³) was calculated as weight of flour (g) divided by flour volume (cm³) [Okaka & Potter, 1979].

Least gelation property

Least gelation property was determined using the method described by Adebowale *et al.* [2005]. Sample suspensions of 2–16% were prepared in distilled water. Each of aliquot dispersion (10 mL) was transferred into a test tube and heated in a boiling water bath for 1 h, cooled rapidly in a cold water bath, and allowed to cool further at 4°C for 2 h. The least gelation concentration was determined when the sample from the inverted test tube did not slip or fall.

Swelling capacity

This was determined with the method described by Leach *et al*. [1959] with modification for small samples. One gram of the flour sample was mixed with 10 mL of distilled water in a centrifuge tube and heated at 80°C for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at $1000 \times g$ for 15 min. The supernatant was decanted and the weight of the paste was taken. The swelling power was calculated as: swelling power = weight of the paste / weight of dry flour.

Sensory evaluation

The formulated samples were made into light gruels, using about 20 g of the sample and 60 mL of water. The reconstituted blends were evaluated along with a traditional complementary food (ogi) and commercial complementary foods (Cerelac). Sensory evaluation was conducted on the reconstituted samples which were coded and presented to 20 untrained panelists (*i.e.*, nursing mothers) who were familiar with the control food samples (*i.e.*, ogi and Cerelac). The sensory evaluation was conducted in a standard sensory laboratory, where each of the panelists was positioned in a separate cubicle to avoid interference. The samples were rated on the following attributes, that is, colour, aroma, taste, mouth feel and overall acceptability using a 9-point hedonic scale range from "like extremely" (9) to "dislike extremely" (1) as described by Ruston *et al*. [1996].

Selection criteria for determining optimal weaning food

A ranking system using six nutritional criteria, *i.e*., protein content, energy value, calcium:phosphorous ratio, total essen-

tial amino acids, biological values and sensory attributes, was devised to determine the optimal blend combination according to the modified method of Griffith *et al*. [1998] (Table 8). Based on the relative importance and interrelationship of those criteria, ranking was reported on an equal weight basis. The weighting of those criteria as to relative importance produced identical conclusive results. The three blends were ranked from 1 to 3 (best to worst) to objectively determine the choice weaning blend. The blend yielding the lowest score was considered to possess the most suitable nutritional characteristics.

Statistical analysis

The data were analysed using SPSS version 15.0. The mean and standard error of means (SEM) of the triplicate analyses of the samples were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means of proximate composition, minerals, antinutritional factors, amino acid compositions, sensory attributes and functional properties; while the means were separated using the new Duncan multiple range test at $p < 0.05$.

RESULTS

Proximate and mineral composition of formulated complementary food samples

The proximate composition of fermented popcorn, African locust bean and bambara groundnut flour blends is presented in Table 1. The moisture content of the food samples ranged from 10.07 ± 0.03 to 10.70 ± 0.73 g/100 g for fermented popcorn-African locust bean-bambara groundnut flour (FPAB) and fermented popcorn-African locust bean (FPA) blend respectively. Protein content of FPAB (26.87 ± 1.07 g/100 g) was significantly higher when compared with fermented popcorn-bambara groundnut (FPB) (20.87 ± 1.02 g/100 g) and FPA (20.49 g/100 g), respectively ($p < 0.05$). For ash content, the values ranged between 0.85 ± 0.01 g/100 g for FPAB and 6.07 ± 1.24 g/100 g for FPA. Fermented popcorn-African locust bean mixes (FPA) was significantly higher when compared with fermented popcorn-bambara groundnut (FPB) and fermented popcorn-African locust bean-bambara groundnut blends (FPAB) ($p < 0.05$). The FPAB sample (2.41 ± 0.18 g/100 g) had the highest value of fiber while FPA had the least. Energy values of FPAB (464.94 ± 1.22 kcal) were higher when compared with the remaining formulated food samples, that is, FPA (432.61 ± 4.97 kcal) and FPB (441.48 ± 3.05 kcal).

Table 2 shows mineral composition of fermented popcorn, African locust bean and bambara groundnut flour blends. Of all the minerals analysed, potassium had the highest value which was significantly higher in FPA (173.75 ± 0.21 mg/100 g) than in the remaining formulated food samples, *i.e.*, FPB (157.45 ± 0.25 mg/100 g) and FPAB (132.75 ± 0.15 mg/100 g) ($p < 0.05$); while copper was the least mineral in the food samples. The Ca/P ratio of the formulated food samples was between 1.41 and 1.73 for FPAB and FPA, respectively. For Na/K ratio, FPB had the least value, while FPAB had the highest value.

Amino acid composition of formulated complementary food samples

The amino acid composition of formulated food samples from fermented popcorn, African locust bean and bambara groundnut flour blends is presented in Table 3. The amino acids profile of FPA showed that glutamic acid (9.17 ± 0.10 mg/100 g) had the highest value, while cysteine (0.68 mg/100 g) had the least value; whereas for FPB glutamic acid (12.13 ± 0.17 mg/100 g) was the highest value and allosocysteine was the least (1.15 ± 0.01 mg/100 g). For the FPAB sample, the amino acid concentrations were between 1.11 ± 0.03 and 12.30 ± 0.20 mg/100 g for glycine and glutamic acid, respectively. Classification of amino acids showed the following range of values: 17.99 and 20.92 mg/100 g for non-essential amino acids (NEAA) with FPA having the highest value and FPAB the least value; 10.82 and 12.18 mg/100 g for conditionally essential amino acids (CEAA) with FPA having the highest values and FPAB the least value; while for essential amino acid (EAA+ histidine+arginine), FPB (27.91 mg/100 g) had the highest value while FPA had the least value.

Calculated nutritional quality of formulated complementary food samples

The calculated nutritional quality of formulated complementary food samples from fermented popcorn, African locust bean and bambara groundnut flour blends is shown in Table 4. Total amino acid (TAA) profile of the food samples range between 52.08 mg/100 g for FPA and 58.94 mg/100 g for FPB. The recommended daily allowance (RDA) percentage met of total essential amino acid (TEAA) was between

TABLE 1. Mean ($\pm$SEM) of macronutrient composition (g/100 g dry weight matter) of fermented popcorn, African locust bean and bambara groundnut flour blends.

Nutrient/Sample	FPA	FPB	FPAB	Ogi	Cerelac	*FAO/WHO (g/100 g)
Moisture	$10.70^a \pm 0.73$	$10.27^{ab} \pm 0.01$	$10.07^{ab} \pm 0.03$	$8.31^b \pm 0.57$	$11.3^a \pm 0.50$	<5
Protein	$20.49^b \pm 1.08$	$20.87^b \pm 1.02$	$26.87^a \pm 1.07$	$6.52^d \pm 0.31$	$15.75^c \pm 0.01$	>15
Fat	$12.76^b \pm 0.20$	$12.31^b \pm 0.11$	$14.59^a \pm 0.15$	$5.17^d \pm 0.11$	$10.53^c \pm 0.02$	10-25
Ash	$6.07^a \pm 1.24$	$2.96^c 0.10$	$4.87^b \pm 0.03$	$1.09^c \pm 0.01$	$3.16^{ab} \pm 0.01$	<3
Fiber	$1.72^a \pm 0.27$	$2.05^a \pm 0.21$	$2.41^a \pm 0.18$	$0.85^b \pm 0.01$	$2.11^a \pm 0.01$	<5
Carbohydrate	$58.95^{cd} \pm 2.41$	$61.78^c \pm 1.01$	$51.27^d \pm 2.11$	$86.38^a \pm 0.21$	$68.42^b \pm 0.01$	64
Energy (Kcal)	$432.61^{bc} \pm 4.97$	$441.48^b \pm 3.05$	$464.94^a \pm 1.22$	$418.08^c \pm 0.47$	$431.58^{bc} \pm 0.01$	400-425

FPA (Fermented popcorn-African locust bean blend; FPB (Fermented popcorn-bambara groundnut blend; FPAB (Fermented popcorn-African locust-bambara groundnut blend. Mean values with the same superscript in a row are not significantly different (P>0.05). *FAO/WHO [1991].

TABLE 2. Mean (±SEM) of mineral composition (mg/100 g) of fermented popcorn, African locust bean and bambara groundnut flour blends.

Nutrient/Sample	FPA	FPB	FPAB	Ogi	Cerelac	*FAO/WHO
Phosphorous	78.95[e]±0.35	88.90[c]±0.21	96.90[b]±0.32	85.95[d]±0.02	400.00[a]±0.01	456
Potassium	173.75[b]±0.21	157.45[c]±0.25	132.75[d]±0.15	102.39[e]±1.01	635.00[a]±0.00	516
Sodium	148.75[a]±0.15	129.30[c]±0.20	127.90[d]±0.31	14.56[e]±0.04	145.00[b]±0.00	296
Calcium	136.91[c]±0.20	139.75[b]±0.15	136.68[c]±0.10	68.66[d]±0.35	600.00[a]±0.01	500
Magnesium	2.86[d]±0.02	3.17[c]±0.03	4.98[b]±0.02	34.91[a]±0.01	0.00[e]	76
Iron	4.14[c]±0.01	4.25[b]±0.03	3.79[d]±0.05	0.26[e]±0.01	7.50[a]±0.01	16
Zinc	2.89[d]±0.05	3.16[c]±0.02	3.83[b]±0.04	0.08[e]±0.00	5.00[a]±0.00	3.2
Copper	1.31[a]±0.02	1.35[a]±0.15	1.38[a]±0.01	0.00[b]	0.00[b]	160
Manganese	2.09[b]±0.02	2.15[b]±0.16	2.65[a]±0.05	0.00[c]	0.00[c]	32
Ca/P	1.73[a]	1.57[b]	1.41[d]	0.79[e]	1.50[c]	-
Na /K	0.86[b]	0.82[c]	0.96[a]	0.14[e]	0.23[d]	-

FPA (Fermented popcorn-African locust bean blend; FPB (Fermented popcorn-bambara groundnut blend; FPAB (Fermented popcorn-African locust-bambara groundnut blend. Mean values with the same superscript in a row are not significantly different (P>0.05). *FAO/WHO [1991].

55.99% for FPA and 82.33% for FPB. The percentage of total essential amino acid results showed that FPAB sample had the highest value (56.50%) and FPA (44.64%) had the lowest value. The percentage difference of leucine and isoleucine in the food samples was between 2.33% for FPB and 38.69% for FPA. The predicted protein efficiency ratio (P-PER) of the formulated food samples showed that FPAB (1.43) had the highest while FPB (0.54) had the lowest value of this ratio. The biological value (BV), essential amino acid index (EAAI) and nutritional index of the food samples ranged between 20.13–39.94%, 29.19–47.38% and 5.98–12.73%, respectively.

Antinutrient composition

Table 5 shows the antinutritional composition of fermented popcorn, African locust bean and bambara groundnut blends. The result showed that oxalate concentration range between 0.015±0.008 mg/100 g for FPAB and 1.925±0.023 mg/100 g for FPA, tannin concentration was within the range of 0.014±0.001 mg/100 g for FPB and 0.026±0.014 mg/100 g for FPA, phytate concentration was between 15.212±0.25 mg/100 g for FPAB and 19.250±2.121 mg/100 g for FPA, while that of trypsin was between the values of 0.018±0.035 mg/100 g and 0.057±0.022 mg/100 g.

Functional properties of formulated complementary foods

Table 6 shows the functional properties of formulated complementary foods compared with control (ogi and Cerelac). Bulk density (BD) of the flour mixes ranged from 0.80 ±0.01g/cm³ to 0.88 ±0.12 g/cm³ for FPB and FPAB, respectively. The values for water absorption capacity (WAC) were between 0.58 ±0.02 mL/g for FPA and 2.34 ±0.12 mL/g for FPAB sample. Swelling capacity (SC) values ranged between 0.53 ±0.17% for FPA and 4.97 ±0.04% for FPB; while that of least gellation (GL) was between 10.5 ±0.70% for FPAB and 12.50 ±0.50% for FPB.

Sensory attributes

The sensory attributes of formulated complementary food samples, ogi (a traditional complementary food) and Cerelac (a commercial formula) is shown in Table 7. The result showed that FPAB food sample was insignificantly higher in terms of aroma, texture and overall acceptability when compared with FPA and FPB samples (p>0.05); similarly, FPB was not significantly higher when compared with those of FPA and FPAB in terms of colour and taste (p>0.05). In comparison, the control food samples, *i.e.*, Cerelac, were significantly higher than the formulated food samples in terms of aroma, colour, taste, texture and overall acceptability (p<0.05).

DISCUSSION

Production of nutrient-rich complementary food is a major problem in Nigeria and other developing countries, due to poor complementation of local food materials such as cereal and legumes.The present study considered this nutritional problem by formulating complementary foods from popcorn, African locust bean and bambara groundnut. The finding showed that the moisture content of the formulated food samples was higher when compared with WHO [1991] recommended value (5%) for complementary foods for older infants and young children. However, the moisture content of the blends was lower, but there was no significant difference between the blend samples and that of Cerelac (p>0.05). In the present study the moisture content of the blends was similar to the finding of Mohamed & Huiming [2007]. Scientific studies reported on the importance of low moisture content in complementary foods, especially to increase the nutrient composition [Amankwah *et al.*, 2009] and shelf life of the product [Kikafunda, 2006; Oyenuga, 1968]. It is evident that the low moisture content of food products inhibits biochemical activities of invading microorganisms, and thereby prevent food spoilage during storage [Kikafunda, 2006].

Protein contents of formulated complementary food samples were significantly higher compared to the control food samples and to FAO/WHO [1991] recommended level. However, the protein content of FPAB sample was the highest when compared with the FPA and FPB samples. This observation could be attributed to the fact that blending of two or more plant-based food materials increases the nutrient

TABLE 3. Amino acid composition (mg/100 g) of formulated food samples from fermented popcorn, African locust bean and bambara groundnut flour blends.

Amino acids	FPA	FPB	FPAB	*RDA
Non-essential amino acids (TNEAA)				
Alanine	2.09[b] ±0.01	2.49[a] ±0.02	2.47[a] ±0.11	-
Aspartic acid	6.30[a] ±0.01	2.51[b] ±0.02	1.30[c] ±0.03	-
Serine	3.36[a] ±0.01	2.22[b] ±0.09	1.92[c] ±0.06	-
Glutamic acid	9.17[b] ±0.10	12.13[a] ±0.17	12.30[a] ±0.20	-
TNEAA	20.92	19.35	17.99	-
Conditionally essential amino acids (TCEA)				
Proline	2.03[a] ±0.01	1.84[ab] ±0.15	1.34[b] ±0.19	-
Glycine	3.10[a] ±0.01	1.46[b] 0.03	1.11[c] ±0.03	-
Arginine	4.27[a] ±0.02	3.97[b] ±0.03	4.42[a] ±0.05	2
Cysteine	0.68[c] ±0.02	1.15[b] ±0.01	1.29[a] ±0.05	-
Tyrosine	2.10[a] ±0.01	3.26[a] ±0.62	2.66[a] ±0.09	-
TCEA	12.18	11.68	10.82	-
Essential amino acids (TEAA)				
Lysine	2.38[c] ±0.01	5.28[a] ±0.10	4.92[b] ±0.04	5.8
Threonine	1.84[b] ±0.01	2.69[a] ±0.04	2.64[a] ±0.07	3.4
Valine	2.69[a] ±0.02	2.72[a] ±0.46	2.60[a] ±0.14	3.5
Methionine	0.84[b] ±0.01	1.29[a] ±0.04	1.47[a] ±0.10	2.2
Isoleucine	1.83[b] ±0.01	4.41[a] ±0.06	4.28[a] ±0.10	2.8
Leucine	4.14[b] ±0.02	4.62[a] ±0.11	4.79[a] ±0.10	6.6
Phenylalanine	2.40[b] ±0.10	3.71[a] ±0.05	3.84[a] ±0.10	2.8
Histidine	1.63[ab] ±0.05	1.74[a] ±0.03	1.51[b] ±0.02	1.9
Tryptophan	1.23[a] ±0.04	1.45[a] ±0.25	1.21[a] ±0.03	1.1
TSAA(Meth+cysteine)	1.52	2.44	2.76	2.5
TArAA (Phenyl+Tyro)	4.5	7.88	7.45	6.3
TEAA	18.98	27.91	27.26	33.9

FPA (Fermented popcorn-African locust bean blend; FPB (Fermented popcorn-bambara groundnut blend; FPAB (Fermented popcorn-African locust-bambara groundnut blend. Mean values with the same superscript in a row are not significantly different (P>0.05). *RDA [FAO/WHO/UNU, 1985].

TABLE 4. Calculated nutritional quality of formulated complementary food samples from fermented popcorn, African locust bean and bambara groundnut flour blends.

Parameters	FPA	FPB	FPAB
TAA (mg/100 g)	52.08	58.94	56.07
RDA% met of TEAAs	55.99	82.33	80.41
TEAA+His+Arg/TAA%	29.15	37.59	37.61
TEAA/TAA%	44.64	54.09	56.50
TNEAA/TAA%	55.36	45.91	43.50
TSAA (Meth+Cys)	1.52	2.44	2.76
TSAA (%)	2.92	4.14	4.92
TArEAA (Phe+Tyr)	4.5	7.88	7.45
TArEAA (%)	8.64	13.34	13.27
TEAA/TNEAA	0.81	1.18	1.30
Leu/Ileu ratio	2.26	1.05	1.12
Leu-Ileu (difference)	2.31	0.21	0.51
Leu-Ileu (%)	38.69	2.33	5.62
P-PER	1.19	0.54	1.43
EAAI (%)	29.19	40.72	47.38
BV (%)	20.13	32.69	39.94
Nutritional index (%)	5.98	8.49	12.73

FPA (Fermented popcorn-African locust bean blend; FPB (Fermented popcorn-bambara groundnut blend; FPAB (Fermented popcorn-African locust-bambara groundnut blend.

TABLE 5. Antinutritional composition (mg/100 g) of fermented popcorn, African locust bean and bambara groundnut blends.

Parameters	Oxalate	Tannin	Phytate	Trypsin
FPA	1.925[ab] ±0.023	0.026[a] ±0.014	19.250[ab] ±2.121	0.057[ab] ±0.022
FPB	0.018[b] ±0.002	0.014[a] ±0.001	18.01[b] ±2.01	0.018[b] ±0.006
FPAB	0.015[c] ±0.008	0.015[a] ±0.005	15.212[b] ±0.25	0.018[b] ±00.035

FPA (Fermented popcorn-African locust bean blend; FPB (Fermented popcorn-bambara groundnut blend; FPAB (Fermented popcorn-African locust-bambara groundnut blend. Mean values with the same superscript in a row are not significantly different (P>0.05).

density of the product [Solomon, 2005; Osman, 2007; Ijarotimi & Olopade, 2009; Oyarekua & Adeyeye, 2009; Eka et al., 2010]. Fat content of FPAB was significantly higher than that of the FPA and FPB samples (p<0.05), and also than the control food samples. Fat content of the food samples met the minimum requirement of 10–25% recommended by WHO for infant food. Comparatively, the fat content of formulated samples was similar to those reported by Lalude & Fashakin [2006] for sorghum and oil – seeds blends (9.87%); and by Amankwah et al. [2009] for fermented maize, rice, soybean and fishmeal blends (8.75–9.38%). The fiber contents of formulated diets were not significantly different from the control food sample (Cerelac); but within the recommended value of FAO/WHO [1991]. The observed low fiber content of these formulated diets would enable the children to consume more

TABLE 6. Functional properties of formulated complementary foods compared with control (ogi and Cerelac).

Parameters	Bulk density (g/cm³)	Water absorption capacity (mL/g)	Swelling capacity (%)	Least gellation (%)
FPA	0.79[ab] ±0.03	0.58[b] ±0.02	0.53[c] ±0.17	11.0[cd] ±1.00
FPB	0.80[b] ±0.01	2.31[a] ±0.20	4.97[a] ±0.04	12.50[bc] ±0.50
FPAB	0.88[a] ±0.12	2.34[a] ±0.12	3.30[ab] ±0.80	10.5[de] ±0.7
Ogi	0.66[c] ±0.01	1.82[a] ±0.02	0.90[c] ±0.03	9.00[e] ±1.11
Cerelac	0.56[d] ±0.03	2.31[a] ±0.21	2.43[bc] ±0.03	14.00[a] ±1.21

FPA (Fermented popcorn-African locust bean blend; FPB (Fermented popcorn-bambara groundnut blend; FPAB (Fermented popcorn-African locust-bambara groundnut blend. Mean values with the same superscript in a row are not significantly different (P>0.05).

TABLE 7. Sensory attributes of fermented complementary food samples, ogi (a traditional complementary food) and Cerelac (a commercial formula).

Parameters	Aroma	Colour	Taste	Texture	Overall acceptability
FPB	6.0[bc]	6.1[bc]	5.3[b]	5.6[c]	5.9[c]
FPA	5.6[c]	6.5[b]	4.8[b]	6.2[c]	5.4[c]
FPAB	6.1[bc]	5.5[c]	5.2[b]	6.5[bc]	6.9[bc]
Ogi	6.7[b]	7.4[a]	7.6[a]	7.3[b]	7.1[b]
Cerelac	8.3[a]	7.7[a]	8.3[a]	8.6[a]	8.4[a]
Range	5.6-8.3	5.5-7.7	4.8-8.3	5.6-8.6	5.4-8.4

FPA (Fermented popcorn-African locust bean blend; FPB (Fermented popcorn-bambara groundnut blend; FPAB (Fermented popcorn-African locust-bambara groundnut blend. Mean values with the same superscript in a row are not significantly different (P>0.05).

of the food samples; and thereby will give the children greater opportunity to meet their daily energy and other vital nutrient requirements [Eka & Edijala, 1972]. The energy values of formulated food samples were more than FAO/WHO [1991] recommended value, but within the range value of ogi (a traditional complementary food) and Cerelac (a commercial formula) respectively. The high energy and protein contents of these complementary foods revealed that they are suitable to support growth and development in infants and young children. Recently, several epidemiological studies have reported that protein-energy malnutrition among children under 5 years of age is increasing in many parts of developing countries due to poor complementary foods, which are low in energy, protein and vital micronutrients that are required for normal growth and development of young children [FMOH, 2005; Power et al., 2005; Galobardes et al., 2006; Lawlor et al., 2006].

The mineral composition of formulated complementary food samples showed that potassium was the most abundant, and this observation was similar to other findings like Olaofe & Sanni [1988] and Oshodi et al. [1999] who reported po-

TABLE 8. Ranking of formulated complementary foods to determine optimal nutritional profile.

Parameters	FPA	FPB	FPAB
Protein (g/100 g)	3	2	1
Energy (kcal)	3	2	1
TAA (mg/100 g)	3	1	2
BV	3	2	1
P-PER	2	3	1
TEAA/TAA%	3	2	1
TEAA+His+Arg/TAA%	3	2	1
RDA% met by TEAA	3	1	2
Ca/P ratio	1	2	3
Overal acceptability	3	2	1
Total Score	27	19	14
Rating	Low	Medium	High

FPA (Fermented popcorn-African locust bean blend; FPB (Fermented popcorn-bambara groundnut blend; FPAB (Fermented popcorn-African locust-bambara groundnut blend. Mean values with the same superscript in a row are not significantly different (P>0.05). TEAA = Total essential amino acids; BV = Biological values, P-PER = predicted protein efficiency ratio, RDA = recommended daily allowance, TAA = total amino acid.

tassium to be the most abundant mineral in Nigerian agricultural products. The calcium:phosphorous ratio of the formulations was observed to be higher than the recommended value of 1.0 [National Research Council, 1989]. This inferred that the diets are suitable to provide the required calcium and phosphorous for the formation of bones and teeth as well as in controlling the level of calcium in blood of the consumers [National Research Council, 1989; Osborne & Voogt, 1978]. The finding also showed that the mineral composition of the diets was low in terms of phosphorous, potassium, sodium, calcium, magnesium, iron and zinc, when compared with Cerelac and FAO/WHO [1991] recommended values for infant formulas. This observation could be attributed to the fact that the formulated food samples are plant based, and besides, commercial formulas are usually fortified with micronutrients in order to meet the FAO/WHO guidelines for infant complementary food formulations. Sodium/potassium (Na/K) ratios of the blends were significantly higher than these of ogi and Cerelac. However, these values were below the recommended value of Trace Element International [1998, 2000], hence, these blends would not affect the nutritional and health wellbeing of the children. A low intake of dietary potassium, especially in the presence of high sodium intake, has been implicated in the pathogenesis of elevated blood pressure (BP) [Morgan, 1982; Ganguli et al., 1990; Institute of Medicine, 2004; Moris et al., 2006]. A World Health Organization study group recommended moderate sodium intake of children. A high sodium intake in early life may condition children to a lifelong high salt appetite [WHO, 1985].

The amino acid profile of the formulated complementary foods showed that glutamic acid was the most abundant. This observation is in close agreement with the report of Olaofe et al. [1994], Oshodi et al. [1998], Adeyeye [2004] and Ogungbenle [2011]. The total amino acids (TAA) of the food samples were far above average and this observation indicates

that the complementary food samples are nutritionally rich to meet the essential amino acids demand of the children. In comparison, the total amino acid profiles of FPB and FPAB were higher compared to melon (53.4g/100 g), pumpkins (38.3g/100 g) and gourd seed (53.6g/100 g protein) respectively as reported by Olaofe *et al.* [1994]; and compared to soybean (44.4g/100 g protein) and pigeon peas (45.2g/100 g protein) as reported by Nwokolo [1987]. The total percentage of essential amino acids (% TEAA) in the food samples was far above average, and this further shows that the formulated food samples were a good source of essential amino acids. The percentage of recommended daily allowance met by essential amino acids of the food samples revealed that the food samples can adequately provide four-fifth of infant and young children daily requirements. The predicted protein efficiency ratios (P-PER) of FPA, FPB and FPAB samples were lower than 2.88 in whole hen's egg [Paul *et al.*, 1976], 2.50 in reference casein [Oyarekua & Eleyinmi, 2004], 4.06 of modified corn ogi [Oyarekua & Eleyinmi, 2004]; but favourably comparable to 1.21 in cowpea, 1.82 in pigeon pea [Salunkhe & Kadam, 1989], 0.27 in sorghum ogi, and 1.62 in millet ogi [Oyarekua & Eleyinmi, 2004]. The essential amino acid index (EAAI) of the food sample was quite low compared with defatted soy flour [Nielsen, 2002]. The essential amino acid index value can be useful as a rapid tool to evaluate food formulations for protein quality. However, it does not account for differences in protein quality due to various processing methods or certain chemical reactions [Nielsen, 2002]. The biological values of formulated diets were better than that of popcorn alone [Ijarotimi & Keshinro, 2011]. This finding could be due to the fact that cereal is deficient in lysine and tryptophan [Davidson *et al.*, 1980]; and that on addition of legumes being rich in tryptophan and lysine but deficient in sulphur-containing amino acids, a desirable pattern of essential amino acids comparable to or higher than the reference protein is obtained [Nnam, 2001]. The use of cereal-legume based food is therefore advocated as an alternative protein and energy source for infant and adult food products [Aykroyd, 1981; Mensah & Tomkins, 2003].

The antinutritional compositions of complementary food samples were reduced to tolerable levels. This indicates that the food product could be utilized effectively since the antinutritional composition has been reduced and there would be no interference with the nutrient like protein and minerals in the food samples. Investigations have reported that fermentation and other processing methods improved the nutritional quality of legumes and cereals by causing significant changes in their chemical composition and elimination of antinutritional factors [Au & Fields, 1981; Yasmine, 2002; Ochanda *et al.*, 2010].

The functional properties of formulated food samples were comparable to the control food samples. Functional properties of food materials are very important for the appropriateness of diet, particularly, for the growing children [Omueti *et al.*, 2009]. The consistency of energy density (energy per unit volume) of the food and the frequency of feeding are also important in determining the extent to which an individual will meet his or her energy and nutrient requirements [Omueti *et al.*, 2009]. The bulk density value is of importance in packaging [Snow, 1974]. The lower loose bulk density implies that less quantity of the food samples would be packaged in constant volume thereby ensuring an economical packaging. However, the packed bulk densities would ensure more quantities of the food samples being packaged, but less economical. Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with immature digestive system [Osundahunsi & Aworh, 2002; Gopaldas & John, 1991]. The water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain [Marero *et al.*, 1988; Mosha & Lorri, 1987]. With respect to water absorption capacity, Giami & Bekeham [1992] reported that the microbial activities of food products with low water absorption capacity would be reduced. Hence the shelf-life of such product would be extended. The swelling capacity is an important factor used in determining the amount of water that food samples would absorb and the degree of swelling within a given time.

Sensory attributes of formulated and control food samples showed that there were significant differences in terms of aroma, colour, taste and texture However, there was no significant difference in the overall acceptability between the FPAB and ogi (p>0.05); while Cerelac was rated significantly higher in terms of the overall acceptability over the formulated food samples (p<0.05). The disparity between the overall acceptability of formulated food samples with that of Cerelac and ogi could be due to the familiarity of the panelists with the ogi and Cerelac over the new formulated products.

CONCLUSION

The study investigated the proximate composition, amino acid profile, sensory attributes and nutritional quality of three formulated complementary foods from the combinations of fermented popcorn, Bambara groundnut and African locust bean flour. The FPAB sample was ranked best when compared with other formulated food samples, *i.e.* FPA and FPB. However, the three formulated samples were good sources of high quality protein of almost adequate or more than adequate of essential amino acids and energy values. Nutritionally, the formulated samples were better than ogi (a traditional complementary food) and comparable to Cerelac (a commercial complementary food) in terms of proximate composition.

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