FORMULATION AND NUTRITIONAL QUALITY OF INFANT FORMULA PRODUCED FROM GERMINATED POPCORN, BAMBARA GROUNDNUT AND AFRICAN LOCUST BEAN FLOUR

Oluwole Steve Ijarotimi[*1], Oluremi Olufunke Keshinro[2]*

Address: [1]Department of Food Science and Technology (Human Nutrition Division), Federal University of Technology, Akure, Nigeria. (Phone: 234-8035670760)

[2] Department of Human Nutrition, Faculty of Public Health, College of Medicine, University of Ibadan, Nigeria (remkesh@yahoo.com)

*Corresponding author: soijarotimi@gmail.com

ABSTRACT

The aim of this present study was to produce and evaluate the nutritional quality of complementary foods from popcorn, African locust bean and Bambara groundnut. The popcorn, bambara groundnut and African locust beans were obtained locally in Akure, Nigeria. The seeds were germinated, oven dried, milled and sieved into flours. The flours were mixed as follows: GPA (70% popcorn, 30% African locust bean), GPB (70% popcorn, 30% bambara groundnut) and GPAB (70% popcorn, 20% bambara groundnut, 10% African locust bean). The chemical composition, functional properties, sensory attributes and nutritional qualities of the food samples were determined using standard methods. The protein content of the food samples range between 23.85±1.54 – 28.84±1.02 g/100g, energy values, 434.47±2.04 - 444.11±2.47 and appreciable amount of minerals. The total essential amino acid (TEAA) composition range from 27.63 to 31.09 g/100g. The calculated biological value range from 29.84 to 42.01 % . The oxalate, tannin, phytate and trypsin concentration of the food samples were reduced; while the choking property of the popcorn-based diets was eliminated with respect to the survival of experimental animals. The calculated molar ratios for [Ca][Phytate]/[Zn], phytate:calcium and phytate:iron were less than the critical values For sensory attribute, the GPB was rated highest in terms of overall acceptability over the

GPA and GPAB, but rated less when compared with ogi and cerelac. It could be concluded that GPB had a better nutritional quality based on the overall ranking using protein, energy, Ca/P ratio, TEAA, biological value and sensory attributes indices.

Keywords: Complementary foods, amino acid profile, nutritional quality

INTRODUCTION

Protein-energy malnutrition among children is the major health challenges in developing countries, particularly Nigeria (**FAO, 2001**). This nutrition problem is ascribed to the inappropriate complementary feeding practices, low nutritional quality of traditional complementary foods and high cost of quality protein-based complementary foods (**Nemer *et al*., 2001; Müller *et al*., 2003; Black *et al*., 2003; FAO 2004; FMOH, 2005; Alozie *et al*., 2009; Eka *et al*., 2010**). The tragic consequences of malnutrition include death, disability, stunted mental and physical growth, and as a result, retarded national socioeconomic development. It is evidence that high prevalence of deaths each year among children aged under five years in the developing world are associated with malnutrition (**WHO, 2002**).The interaction of poverty, poor health and poor complementary feeding practices has a multiplier effect on the general welfare of the children population and also contributes significantly towards growth retardation, poor cognitive development, illness and death amongst children in developing countries, particularly Nigeria (**Pollit, 1994; Duncan *et al*., 1994; Kretchmer *et al.*, 1996; Bhattacharya *et al.*, 2004; Anigo *et al*., 2007**). It is well known that high cost of fortified nutritious proprietary complementary foods in many parts of developing countries is always beyond the reach of most families (**Muhimbula *et al*., 2011**); hence many families depend on inadequately processed and low quality traditional complementary foods to wean their children.

The complementary feeding period refers to the stage of life when foods and/or liquid milks are fed to infants and young children in addition to breast milk; non-breast-milk food items consumed at this time are defined as complementary foods. Complementary foods may be either prepared specially for the young child, both to meet age-related nutritional needs and to mitigate immaturity in chewing and swallowing, or they may be selected from the same foods consumed by other members of the family (**Brown 1998**). The complementary feeding usually begins at 6 months and continues up to the age of 24 months when transition from exclusive breastfeeding to semi-solid foods begins. It is at this stage that the nutritional

requirements of many infants are not met, thus leading to the onset of malnutrition that is prevalent in children under 5 years of age worldwide (**Daelmans and Saadeh, 2003; Anigo *et al.*, 2009**). Anigo *et al.* (**2009**) and Jansen (**1992**) reported high prevalence of malnutrition in children in the zone with introduction of plant-based complementary foods at much earlier age than the 6th month recommended by World Health Organisation. Study has shown that plant-based complementary foods are insufficient to meet the needs for certain nutrients (**Dop and Benbouzid, 1999**).

The traditional complementary foods in Nigeria are cereal based (e.g, ogi) and other family diets (cassava, yam, rice, amala, etc); and these plant-based complementary foods are not beneficial to the growth and development of the children (**Nemer *et al.*, 2001; Müller et al., 2003; Black *et al.*, 2003; FAO 2004; NPC/ICFM, 2009, Eka *et al.*, 2010**). For instance, investigations have shown that ogi (corn gruel, a traditional complementary food) and other family diets often fail to meet the nutritional needs of the infant due to poor nutritive values (**Solomon 2005; Fernandez *et al,* 2002**); hence, they have been implicated in the aetiology of protein–energy malnutrition in the community where they are solely used as the complementary food (**Okoye 1992; Devlin 1997**). In view of the nutritional problem that associated with traditional complementary foods, the present study, therefore, aim at formulating complementary foods from popcorn (*Zea mays* everta), African locust beans *(Parkia biglobosa*) and bambara groundnut (*Vigna subterranea L.*) flour. The use of cereal-legume based food has long been advocated as alternative protein and energy source for infant and young children food products (**Aykroyd, 1981; Mensah an Tomkins, 2003**). It is evident that when cereals and legumes are judiciously selected and combine a desirable pattern of essential amino acids of high biological value is obtained (**Nnam, 2001**). Cereals are deficient in essential amino acids like lysine and tryptophan (**Davidson *et al.*, 1980**). While, legumes are deficient in sulphur containing amino acids, that is, methionine and cystine, but rich in tryptophan and lysine.

The aim of this work was to produce and evaluate nutritional quality of complementary foods from the combination of popcorn, African locust bean and Bambara groundnut. These food materials were purposely selected, because of their availabilities locally and also to complement one another to obtain a balanced amino acid profile.

MATERIAL AND METHODS

Sources of food materials

The popcorn, African locust bean and Bambara groundnut seeds were obtained from a local market; while the commercial formula (Cerelac) was obtained from a NAO Supermarket in Akure city, Ondo State, Nigeria.

Food processing: germinated popcorn, bambara groundnut and African locust beans flour

Popcorn: The popcorn seeds were sorted pretreated for 5 min with 200 ppm of bleach containing 5.25% sodium hypochlorite, mixed in deionized water to control microbial growth (Hsu *et al.*, 1980). Seeds were rinsed, soaked in deionized water (1:3, w/v) for 9 hr at ambient temperature (23–25°C). Seeds were drained and placed on perforated aluminum pans lined with filter paper, then placed in a dark, temperature controlled cabinet at 30°C for germination.After 4 days the seeds were germinated and the germinated seeds were washed with distilled water manually, oven dried at 60°C (Plus11 Sanyo Gallenkamp PLC, UK) for 20 hours, milled using a Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard). The popcorn flour was packed in plastic container sealed with aluminum foil and stored at room temperature (27°C) prior to analyses.

Bambara groundnut: The Bambara groundnut seeds were sorted and pretreated for 5 min with 200 ppm of bleach containing 5.25% sodium hypochlorite, mixed in deionized water to control microbial growth (Hsu et al., 1980). Seeds were rinsed, soaked in deionized water (1:3, w/v) for 24 hr at ambient temperature (23–25°C). Seeds were drained and placed on perforated aluminum pans lined with filter paper, then placed in a dark, temperature controlled cabinet at 30°C for germination. The seeds germinated after 7 days and the seeds were washed with distilled water manually, oven dried at 60°C (Plus11 Sanyo Gallenkamp PLC, UK) for 20 hours, milled using a Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard). The sample was stored at room temperature (27°C) in a well sealed plastic container prior to analyses.

African locust bean: The African locust beans were sorted and pretreated for 5 min with 200 ppm of bleach containing 5.25% sodium hypochlorite, mixed in deionized water to control microbial growth (Hsu et al., 1980). Seeds were rinsed and then soaked in deionized water (1:3, w/v) for 24 hr at ambient temperature (23–25°C). Seeds were drained and placed on perforated aluminum pans lined with filter paper, then placed in a dark, temperature controlled cabinet at 30°C for germination. The seeds germinated after 7 days. The germinated seeds were washed with distilled water manually, oven dried at $60^{o}C$ (Plus11 Sanyo Gallenkamp PLC, UK) for 20 hours, milled using a Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard). The sample was stored at room temperature $(27^{o}C)$ in a well sealed plastic container prior to analyses.

Food formulations

Nutrisurvey linear programming (2004) software was used to determine the proportion of popcorn, African locust bean and Bambara groundnut flour to be blended with reference to protein requirement of infants (18 g/day). The flour of food samples were blended in the following proportions, that is, 70% of germinated popcorn and 30% of germinated Africa locust bean to obtain GPA blend and 70% of germinated popcorn and 30% of germinated Bambara groundnut flour to obtain GPB blend; while 70% popcorn, 20% Bambara groundnut and 10% African locust bean flour were blended to obtain GPAB sample. The ogi (corn gruel and a traditional complementary food) and Cerelac (a commercial complementary formula) were used as the control food samples.

Proximate Analyses

Proximate analysis was carried out on the raw, germinated and fermented African locust bean flour. The moisture content was determined using AOAC (2005), protein was determined by micro-Kjeldahl using the Tecator Digestion System and Kjeltec Auto 1030 Analyzer (Tecator AB, Sweden). Fat was determined by ether extraction using the Soxtec System HT method (Tecator Soxtec System HT 1043 Extraction Unit, Tecator AB, Sweden). Ash was determined by AOAC (2005) method. 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 carbohydrate with the recommended factors (2.44, 8.37 and 3.57 respectively) as proposed by Martin and Coolidge [1978].

Mineral Analyses

The method described by Association of Official Analytical Chemists (AOAC) (**2005**) was used for mineral analysis. The samples were ashed at 550°C. The ash was boiled with 10ml of 20% hydrochloric acid in a beaker and then filtered into a 100ml 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 and 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]. All values were expressed in mg/100g.

Amino Acid Determination

Sample preparation for amino acid analysis: About 2.5.0 g of each sample were weighed into the extraction thimble and the fat extracted with chloroform/methanol (2:1v/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 was weighed into glass ampoules. Seven milliliters of 6 M 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 $\pm5°$C for 22 h. The ampoule was allowed to cool; the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in a plastic specimen bottle, and 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 hydrolyzed in 4.67M KOH containing 1% w/v thiodiglycol for 18hrs at 110°C. After hydrolysis the KOH was neutralized with 2.4M perchloric acid, and the supernatant was adjusted to pH 3.0 with acetic acid. A 20μL 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:

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

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. In this present study tryptophan was not considered in the determination of EAAI.

Nutritional index of the food samples were calculated using the formula below:

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

Biological value was calculated according to Oser (**1959**) cited by Mune *et al*. (**2011**) using the following equation:

$$BV = 1.09 \times \text{Essential amino acid index [EAAI]} - 11.7$$

The Protein Efficiency Ratio [PER] was estimated according to the regression equations developed by Alsmeyer *et al*. (1974) cited by Mune *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 flour sample with 3% trichloro-acetic acid by shaking at room temperature followed by high speed centrifugation as described by Wheeler and Ferrel (**1971**). This method depends on an iron to phosphorus ratio of 4: 6. Five grams of the test sample was extracted with 3% tri-chloro acetic 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 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 99.9% methanol for 20 min at room temperature with constant agitation. After centrifugation for 10 min at 653 x g, 5 ml of vanillin-HCl [2% vanilli and 1% HCl] reagent was 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.

Oxalate content determination: Oxalate was determined by AOAC (**2005**) method. 1 g of the sample was weighed into 100 ml conical flask. 75 ml of 3 M H2SO4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1h 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 KMnO4 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 [1g each] were extracted continuously at ambient temperature for 3 h with 50 mL, 10 mM 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 M NaOH. After extraction, the suspension was shaken and diluted with distilled water such 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]

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

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

Choking property determination

Fifteen male and female albino rats of the Wistar strain, weaned at 21 days, were obtained from the disease-free stock of the central animal house of College of Medicine, University of Ibadan, and reared on a balanced commercial stock diet (Pfizer Livestock Feed Ltd, Ikeja, Nigeria) until they were 30 days old and allocated on the basis of weight and litter origin to three groups of five rats each. They were individually housed in perforated Perspex cages. The three groups of animals were fed with pelleted germinated food samples (GPA, GPB and GPAB) respectively for 28 days. The mean of survival periods were calculated for the groups of animals as follows:

Mean of survival period = $\underline{\text{Cumulative number of survival albino rats for 28 days}}$

Number of albino rats per group (5)

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 x g for 30 min. The volume of water or oil on the sediment water measured. 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 cm^{-3}) was calculated as weight of flour [g] divided by flour volume (cm^3) **(Okaka and Potter, 1979)**.

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 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×g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as follow:

swelling power = weight of the paste / weight of dry flour

Least gelation: Least gelation property was determined using the method described by Coffman and Garcia (**1977**). Sample suspensions of 2 – 16% were prepared in distilled water. 10 ml of each of the prepared dispersions was transferred into a test tube and heated in a boiling water bath for 1 hour, cooled rapidly in a cold water bath, and allowed to cool further at 4^{0}C for 2 hours. The least gelation concentration was determined when the sample from the inverted test tube did not slip or fall.

Sensory evaluation: The formulated samples were made into light gruels, using about 20 g 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 (ogi and cerelac). The sensory evaluation was conducted in a well 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 9 point hedonic scale as follows:

9 = like extremely

8 = like very much

7 = like moderately

6 = like slightly

5 = neither like nor dislike

4 = dislike slightly

3 = dislike moderately

2 = dislike very much

1 = dislike extremely

Statistical analysis

The data were analysed using SPSS version 16.0. 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 AND DISCUSSION

Macronutrient and mineral composition of formulated food samples

The macronutrient and mineral composition of the formulated food and control food samples are presented in Tables 1 and 2 respectively. The moisture content values of the formulated food samples range between 5.7±0.1 and 10.2±1.8 g/100g for the germinated popcorn:bambara groundnut flour (GPB) and popcorn:African locust bean flour (GPA) respectively. The moisture content of GPB blend (5.7±0.1 g/100g) was lower when compared with other formulated food samples, that is, germinated popcorn:African locust bean:bambara

groundnut (GPAB) (6.3±0.1 g/100g) and GPA (10.2±1.8 g/100g) blend, and the control food samples, that is, ogi (8.3±0.6 g/100g) and Cerelac (11.3±0.5 g/100g). The lower moisture content of GPB and GPAB samples is a desirable phenomenon, as it will enhance the keeping quality of the samples since water for microbial activity is low. Scientific investigation has reported that low moisture content in food samples increased the storage periods of the food products (**Alozie *et al*., 2009**); while high moisture content in foods encourage microbial growth; hence, food spoilage (**Temple *et al*., 1996**).

The protein content of GPAB sample (28.8±1.0 g/100g) was higher than GPB (28.4±1.1 g/100g) and that of GPA (23.9±1.5 g/100g) respectively. This observation could be attributed to the supplementation of the popcorn based complementary food sample with two different legumes, that is, bambara groundnut and African locust bean flour. However, the protein contents of experimental food samples were significantly higher when compared with the ogi (a traditional complementary food) and cerelac (a commercial formula) samples. Investigations have shown that protein content of cereal- legume combination (i.e., two or more plant-based food materials) is better than those produced from cereal (i.e., a single plant based food materials) (**Solomon, 2000; Achi, 2005; Wakil and Onilude, 2009**). The energy values of experimental food samples range between 434±2.0 kcal for GPA and 444±2.1 kcal. for GPB and these energy values were significantly higher when compared with the traditional complementary food (i.e., ogi) (p<0.05), but they were within the range of Cerelac energy value (432±0.1 kcal.).

Nutritionally, the protein contents and energy values of the experimental food samples met the FAO/WHO (1991) specification guidelines for the young child complementary food formulations. In comparison, the nutrient-dense of formulated food samples was higher than that of the traditional complementary foods that characterize with low energy and nutrient density (**King and Ahworth, 1987**) and they were comparable to the commercial formulas (e.g. Cerelac). Hence, it could be deduced that the formulated food samples were better than ogi, which has been implicated in the eatiology of malnutrition among children who were solely weaned on ogi (**Okoye 1992; Devlin 1997; Mohamed and Huiming, 2007**). Epidemiological study has investigated that malnutrition constitutes a serious nutritional and health problem for children between 6 to 18 months of age, the period of complementary feeding, in Nigeria and other developing countries, due to poor complementary feeding practices (Daelmans and Saadeh, 2003). This nutrition problem is responsible for growth retardation, increase in morbidity and mortality rate among children falling within the low-

income families who cannot afford the high cost of fortified nutritious proprietary complementary foods (**Traoré, 2005; Bruyeron *et al*., 2010; Muhimbula *et al*., 2011**).

Table 1 Mean (± SEM) of macronutrient composition (g/100g Dry weight matter) of germinated popcorn, African locust bean and bambara groundnut blends flour blends

Nutrient/Sample	GPA	GPB	GPAB	Ogi	Cerelac	*RV (g/100g)
Moisture	10.2^a ±1.8	5.7^b ±0.1	6.3^b ±0.1	8.3^{ab} ±0.6	11.3^a ±0.5	<5
Protein	23.9^b ±1.5	28.4^a ±1.1	28.8^a ±1.0	6.5^d ±0.3	15.8^c ±0.1	>15
Fat	11.7^a ±0.3	12.1^a ±0.3	9.9^b ±0.1	5.2^c ±0.1	10.5^b ±0.2	10-25
Ash	1.9^d ±0.1	2.7^c ±0.1	2.5^b ±0.1	1.1^e ±0.0	3.2^a ±0.1	<3
Fiber	1.7^a ±0.2	1.8^a ±0.1	1.2^b ±0.1	0.9^b ±0.1	2.1^a ±0.2	<5
Carbohydrate	60.8^c ±3.8	55.4^d ±2.0	57.5^{ab} ±3.0	86.4^a ±0.2	68.4^b ±0.1	64
Energy (Kcal.)	444^a ±2.5	444^a ±1.1	434^b ±2.0	418^c ±0.5	432^b ±0.1	400-425

Legend: Data were analysed on triplicates; Mean values with the same superscript in a row are not significantly different (P>0.05).

*RV (*Recommended values (g/100g); GPA (Germinated popcorn-African locust bean blend; GPB (Germinated popcorn-bambara groundnut blend); GPAB (germinated popcorn-African locust-bambara groundnut blend); *(CODEX CAC/GL 08. 1991): Codex alimentarius: Guidelines on formulated supplementary foods for older infants and young children.*

The mineral composition of the formulated food sample presented in Table 2 showed that potassium was the highest mineral in GPA (496±0.2 mg/100g), GPB (564±0.2 mg/100g) and GPAB (387±0.1 mg/100g), while manganese was the least in GPA (1.7±0.0 mg/100g) and 1.7±0.2 mg/100g) in GPAB, while copper (1.7±0.2 mg/100g) was the least in GPB sample. In comparisons, the mineral contents of the formulated food samples were higher when compared with the traditional complementary food sample (ogi), but lower than that of cerelac and FAO/WHO (**1991**) recommended values. The variation in mineral content of formulated food samples with that of cerelac (a commercial formula) could be due to the enrichment of the cerelac product with essential mineral during production. The ratio of Ca/P of the food samples range between 1.9 for GPAB and 2.7 for GPAB. This observation

indicates that the formulated food samples would serve as good sources of minerals such as calcium and phosphorous, which are considered essential for bone and teeth formation and development in children. It is evidence that food products containing a Ca/P ratio of >1.0 is rated good, while <0.5 is rated poor (Nieman *et al.*, 1992). The ratio of Na/K of the food samples range between 0.24 for GPB and 0.37 for GPAB. This indicates that the formulated food sample were suitable as complementary food for infants with immature heart. Potassium has a beneficial effect on sodium balance. A high intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risks (**Langford 1983; Cappuccio and McGregor, 1991).** Hence, the sodium to potassium (Na/K) ratio in the body is of great concern for the prevention of high blood pressure. A Na/K ratio less than one is recommended in the diets of people who are prone to high blood pressure and similarly for children with immature heart (**Langford 1983; Cappuccio and McGregor, 1991**).

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

Nutrient/Sample	GPA	GPB	GPAB	Ogi	Cerelac	*RV
Phosphorous	86.7[c] ±0.2	77.8[e] ±0.2	88.8[b] ±0.2	86.0[d] ±0.02	400[a] ±0.1	456
Potassium	496[c] ±0.15	564[b] ±0.2	387[d] ±0.1	102[e] ±1.0	635[a] ±0.01	516
Sodium	153[a] ±0.3	136[c] ±0.3	142[b] ±0.2	14.6[d] ±0.1	145[b] ±0.0	296
Calcium	175[b] ±0.2	213[a] ±0.2	170[c] ±0.2	68.7[d] ±0.4	600[e] ±0.1	500
Magnesium	3.9[c] ±0.2	3.2[d] ±0.1	4.3[b] ±1.1	34.9[a] ±0.1	0.0	76
Iron	5.8[a] ±0.2	4.8[b] ±0.1	5.9[a] ±0.1	0.3[d] ±0.1	7.5[c] ±0.0	16
Zinc	3.6[b] ±1.1	2.8[b] ±0.1	2.7[b] ±0.1	0.1[e] ±0.0	5.0[a] ±0.0	3.2
Copper	2.3[a] ±0.2	1.7[b] ±0.2	2.4[a] ±1.5	0.0	0.00	160
Manganese	1.7[b] ±0.0	2.2[a] ±0.1	1.7[b] ±0.1	0.0	0.0	32
Iodine (µg)	-	-	-		80	-
Ca/P	2.0	2.7	1.9	0.8	1.5	-
Na /K	0.3	0.2	0.4	0.1	0.2	-

*(-) Not detected, Data were analysed on triplicates, Mean values with the same superscript in a row are not significantly different (P>0.05). *(RV) Recommended values (g/100g) GPA) (CODEX CAC/GL 08. 1991): Codex alimentarius: Guidelines on formulated supplementary foods for older infants and young children.*

Amino Acid Profile and predicted nutritional quality of the formulated complementary food samples

The amino acid profile and nutritional quality of the formulated complementary food samples are presented in Tables 3 and 4. The result showed that the non essential amino acids content of the formulated food samples range between 3.6±0.4 mg/100g for serine and 15.4±0.8 mg/100g for glutamic acid in GPA, 2.3±0.01 mg/100g for alanine and 13.14±0.0 mg/100g for glutamic acid in GPB and 2.7±0.1 mg/100g for alanine and 13.01±0.3 mg/100g for glutamic acid in GPAB. For the conditionally essential amino acids (TCEA), the values range between 1.7±0.2 mg/100g for cysteine and 3.9±0.8 mg/100g for arginine in GPA, 1.4±0.0 mg/100g for cysteine and 4.2±0.1 mg/100g for arginine in GPB and 1.6±0.1 mg/100g for glycine and 3.6±0.1 mg/100g for tyrosine. The essential amino acid values range between 0.8±0.0 mg/100g for methionine and 5.6±0.1 mg/100g for leucine in GPA, 1.2±0.1 mg/100g for tryptophan and 4.7±0.1 mg/100g for valine in GPB and 0.9±0.0 mg/100g for tryptophan and 4.6±0.1 mg/100g for phenylalanine in GPAB sample. Nutritionally, the recommended daily allowances (RDA) of some of the essential amino acids (valine, isoleucine and phenylalanine) were adequately met by the formulated food samples (**FAO/WHO, 1991**).

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

Amino acids	GPA	GPB	GPAB	*RDA
Non essential amino acids (TNEAA)				
Alanine	4.4[a]	2.3[b]	2.7[b]	-
	±0.3	±0.1	±0.1	
Aspartic acid	5.2[a]	3.2[b]	3.4[b]	-
	±0.9	±0.1	±0.3	
Serine	3.6[a]	2.9[b]	2.7[c]	-
	±0.4	±0.1	±0.0	
Glutamic acid	15.4[a]	13.1[b]	13.0[b]	-
	±0.8	±0.0	±0.3	
Conditionally essential amino acids (TCEA)				
Proline	3.9[a]	1.7[b]	1.8[b]	-
	±0.2	±0.1	±0.2	
Glycine	3.5[a]	1.7[b]	1.6[b]	-
	±0.1	±0.1	±0.1	
Arginine	3.9[a]	4.2[a]	3.3[b]	2
	±0.8	±0.1	±0.1	

Cysteine	1.7[a]	1.4[b]	1.7[a]	-
	±0.2	±0.0	±0.0	
Tyrosine	2.3[b]	3.6[a]	3.6[a]	-
	±0.3	±0.2	±0.1	
Essential amino acids (TEAA)				
Lysine	5.1[a]	4.6[b]	4.4[b]	5.8
	±0.4	±0.1	±0.1	
Threonine	2.3[c]	3.5[a]	2.8[b]	3.4
	±0.2	±0.2	±0.1	
Valine	3.8[b]	4.7[a]	4.0[b]	3.5
	±0.0	±0.1	±0.0	
Methionine	0.8[a]	1.5[a]	2.0[a]	2.2
	±0.0	±0.1	±0.5	
Isoleucine	3.3[a]	3.2[a]	3.1[a]	2.8
	±0.1	±0.1	±0.1	
Leucine	5.6[a]	4.1[b]	3.7[b]	6.6
	±0.1	±0.2	±0.1	
Phenylalanine	3.6[b]	4.6[a]	4.8[a]	2.8
	±0.1	±0.0	±0.2	
Histidine	1.7[b]	3.6[a]	3.6[a]	1.9
	±0.2	±0.0	±0.1	
Tryptophan	1.4[a]	1.2[ab]	0.9[b]	1.1
	±0.1	±0.1	±0.0	
TSAA(Meth+cystein)	2.5	2.9	3.7	2.5
TArAA (Phenyl+Tyro)	5.9	8.2	8.3	6.3
TEAA	27.6	31.1	29.1	33.9

*Data were analysed on duplicates, Mean values with the same superscript in a row are not significantly different [P>0.05]; *source of RDA: FAO/WHO. 1991.*

The result of nutritional quality of the food samples (Table 4) showed that the percentage RDA met of essential amino acids range between 81.5% for GPA and 91.7% for GPB. The ratio of total essential and non-essential amino acids showed that the value range between 0.6 for GPA and 0.9 for GPAB. The values of protein efficiency ratio of the food samples were 1.8, 1.7 and 0.85 for the GPA, GPAB and GPAB respectively. The essential amino acid indices (EAAIs) of the GPA, GPAB and GPAB food samples were 38.1%, 45.6% and 49.3% respectively; while that of the biological values (BV) were 29.84% for the GPA, 38.0% GPB and 42.0% GPAB sample. Generally, a protein material is said to be of good nutritional quality when its biological values (BV) is high (70-100%) and also when the essential amino acid index (EAAI) is above 90% and to be useful as food when the values is around 80% and to be inadequate for food material when below 70% (Oser, 1959). In

comparison, the BV and EAAI values in this present study were quite low relatively to the values reported by the Oser (**1959**); and these findings could be attributed to the complex metabolic process during which the lipids, carbohydrates, and storage proteins within the seed are broken down in order to obtain the energy and amino acids necessary for the plant's development (**Ferreira *et al*., 1995; Jachmanian et al., 1995; Podesta and Plaxton, 1994; Ziegler, 1995**). However, it is observed that the BV and EAAI values of the formulations were higher when compared with the popcorn flour sample earlier reported by Ijarotimi and Keshinro (**2011**). Davidson et al. (**1980**) reported that cereal is deficient in lysine and tryptophan; and that on addition of legumes that rich in tryptophan and lysine but deficient in sulphur containing amino acid, 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 alternative protein and energy source for infant and adult food products (**Aykroyd, 1981; Mensah and Tomkins, 2003**).

Table 4 Calculated nutritional quality of formulated food samples

Parameters	GPA	GPB	GPAB
TAA[mg/100g]	43.6	36.2	33.6
RDA% met of TEAAs	81.5	91.7	85.9
TEAA+His+Arg/TAA%	33.2	38.9	36.0
TEAA/TAA%	38.8	46.2	46.4
TNEAA/TAA%	61.2	53.8	53.6
TSAA[Meth+Cys]	2.5	2.9	3.7
TArEAA [Phe+Tyr]	5.9	8.2	8.3
TEAA/TNEAA	0.6	0.9	0.9
PER	1.8	1.7	0.9
EAAI [%]	38.1	45.6	49.3
BV [%]	29.8	38.0	42.0
Nutritional index [%]	9.1	13.0	14.2

Antinutrient composition and choking properties of popcorn-based food product

Antinutrient composition of popcorn-based complementary food samples is presented in Table 5. The oxalate concentration in the food samples range between 0.15±0.03 mg/100g for GPB and 2.14±1.04 mg/100g for GPA, tannin content was between 0.03±0.01 mg/100g for GPA and 0.51±0.38 mg/10g for GPAB, phytate was between 15.31±0.17 mg/100g for GPB and 32.90±2.47 mg/100g for GPAB, while trypsin inhibitor was between 0.06±0.02

mg/100g and 0.12±0.01 mg/100g for GPA and GPB respectively. The concentrations of these antinutritional factors were within the tolerable level. It is evident that cooking and germination processing methods improve the nutritional quality of food products by reducing or eliminating the antinutritional factors in food products (**Oboh *et al.*, 2000; Mbithi-Mwikya *et al.*, 2001; Ibrahim *et al.*, 2002; Syed *et al.*, 2011**).

Table 5 Antinutrient composition (mg/100g) of germinated and germinated popcorn, African locust bean and Bambara groundnut blend

Parameters	Oxalate	Tannin	Phytate	Trypsin
GPA	2.14^a	0.03^a	21.41^b	0.06^{ab}
	±1.0	±0.01	±1.82	±0.02
GPB	0.15^{ab}	0.12^a	15.31^c	0.12^a
	±0.03	±0.1	±0.17	±0.01
GPAB	0.32^{ab}	0.51^a	32.9^a	0.09^{ab}
	±0.19	±0.38	±2.5	±0.03

Data were analysed in triplicates. Mean values with the same superscript in a row are not significantly different [P>0.05]

The mean survival period of albino rats fed with germinated popcorn flour showed that all the animals were survived throughout the experimental periods. This observation showed that germination processing methods eliminate or reduce to tolerable level the chemical responsible for the choking properties of popcorn, which formed large proportion of the formulated food samples. Investigations have reported that germination and other processing methods improved on the nutritional quality of legumes and cereals by causing significant changes in chemical composition and elimination of antinutritional factors (Bau *et al.*, 1997; Mohamed *et al.*, 2007; Syed *et al.*, 2011).

Calculation of phytate and zinc, iron and calcium molar ratios to predict their bioavailability

The molar ratios of phytate and zinc, iron and calcium to predict their bioavailability are shown in Table 6. The phytate:zinc molar ratio range between 0.546 mol/kg. for GPB and 1.21 mol/kg. for GPAB, phytate:calcium molar ratio values range between 0.004 mol/kg. for GPB and 0.012 mol/kg. for GPAB, (calcium)(phytate):zinc was between 2.59 mol/kg. for GPA and 5.14 mol/kg. for GPAB, while that of phytate:iron was between 0.27 mol/kg. for GPB and 0.48 mol/kg. for GPAB. It was observed in this study that the bioavailability of zinc,

iron and calcium in GPB, and GPA were higher when compared with the GPAB. However, the values of Phytate:Zinc, Phytate:Calcium, (Ca)(Phytate):Zinc and Phytate:Iron molar ratios were lower than the critical values reported by other investigators (**Morris and Ellis, 1985; Davies *et al*., 1985; Bindra *et al*., 1986; Gibson *et al*., 1991; Gibson 2006**). The inhibitory effect of phytate on zinc, iron and calcium absorption has been quantified by the molar ratios of phytate to zinc, iron and calcium in the diet. Ratios greater than15.0, 0.24, 200 and 1.0 have been associated with biochemical and/or clinical evidence of zinc calcium and iron deficiency (**Morris and Ellis, 1985**; **Davies *et al*., 1985; Bindra *et al*., 1986; Gibson *et al*., 1991; Gibson 2006**). It is well known that zinc, iron and calcium are essential trace elements for human nutrition (**Kono and Yoshida 1989**). It is well known that children are more vulnerable to sub-optimal zinc, iron and calcium status with adverse effects on their growth rate and cognitive development (**Hambidge *et al*., 1985**), presumably because of their high zinc, iron and calcium requirements for growth (**Kono and Yoshida 1989**). The importance of a foodstuff as a source of dietary zinc, iron and calcium depends upon both the zinc contents of these minerals in food products and the level of other constituents in the diet that affect their bioavailability. Phytic acid may reduce the bioavailability of dietary zinc, iron and calcium by forming insoluble mineral chelate at physiological pH.

Table 6 Relationship between phytate and bioavailability of selected minerals (zinc, iron and calcium) (mol/kg)

Parameters	Phytate:Zinc	Phytate:Calcium	(Ca)(Phytate):Zinc	Phytate:Iron
GPA	0.59	0.007	2.59	0.32
GPB	0.55	0.004	2.90	0.27
GPAB	1.21	0.012	5.14	0.48
*Critical values	>15.0	>0.24	>200	>1.0

Sources: *phytate: calcium > 0.24 (Morris & Ellis, 1985), phytate : iron > 1 (Hallberg et al., 1989), phytate : zinc >15 (Turnlund et al.,1984; Sandberg et al., 1987; Morris & Ellis, 1989), phytate : calcium/zinc > 200 (Davies et al., 1985; Bindra, et al., 1986; Gibson 2006)*

Functional properties of the food samples

The functional properties of formulated food samples and control food samples (ogi and cerelac) are shown in Table 7. Results showed that the swelling capacity of the food samples ranged between 0.67±0.03 and 4.22±0.01 for GPA and GPB sample respectively.

Bulk density ranged between 0.71±0.01 and 0.82±0.03 for GPAB and GPA sample respectively; while water absorption capacity ranged between 0.67±0.03 and 4.22±0.01 for GPA and GPB respectively; for the least gellation, the value range between 11.50±0.05 and 16.00±2.01 for GPB and GPA respectively. The functional properties of the formulated food samples were compared with ogi and cerelac sample. It was observed that there was significant difference between the bulk density, swelling capacity and least gellation of formulated food samples and control food samples (ogi and cereal (p<0.05). However, there was no significant different between the formulated food samples and control in term of water absorption capacity (p>0.05).

The functional properties of the food materials are very important for the appropriateness of the 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 and Aworh, 2002; Gopaldas and 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 and Lorri, 1987**). With respect to water absorption capacity, Giami and 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. The present study showed that the swelling capacities of the GPAB and cerelac were not significantly different.

Table 7 Functional properties of formulated complementary foods compared with control (ogi and cerelac)

Parameters	Bulk Density	Water absorption capacity	Swelling Capacity	Least Gellation
GPA	0.82[a]	2.19[a]	0.67[c]	16.00[a]
	±0.03	±0.19	±0.03	±2.01
GPB	0.78[ab]	1.96[a]	4.22[a]	11.5[cde]
	±0.01	±0.02	±0.01	±0.05
GPAB	0.71[bc]	2.04[a]	2.45[b]	14.50[ab]
	±0.11	±0.08	±0.75	±1.50
Ogi	0.66[c]	1.82[a]	0.90[c]	9.00[e]
	±0.01	±0.02	±0.03	±1.11
Cerelac	0.56[d]	2.31[a]	2.43[b]	14.00[abc]
	±0.03	±0.21	±0.03	±1.21

Data were analysed on triplicate. Mean values with the same superscript in a row are not significantly different [P>0.05]

Sensory attributes of the formulated food samples and control food samples

The sensory attributes of formulated food samples, ogi (a traditional complementary food) and cerelac are shown in Table 8. The aroma, colour, taste, texture and overall acceptability parameters were considered by the nursing-mother panelists. The result showed that there were significant difference between the aroma, colour, taste and texture of formulated food samples when compared with the control food samples (i.e., ogi and cerelac). However, there was no significant different between the overall acceptability of the formulated food samples and that of ogi (p>0.05); while cerelac was significantly rated 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 and that of cerelac and ogi could be due the familiarity of the panelist with the ogi and cerelac over the new formulated products; and besides, it has been scientifically reported that germination technique negatively affect the organoleptic properties of food products (**Nnanna *et al.*, 1990; Bau *et al.*, 2000; Uwaegbute *et al.*, 2000**)

Table 8 Sensory attributes of germinated complementary food samples, ogi (a traditional complementary food) and cerelac (a commercial formula)

Parameters	Aroma	Colour	Taste	Texture	Overall acceptability
GPB	5.3[c]	6.3[b]	5.9[b]	5.8[c]	6.5[b]
GPA	5.0[c]	6.3[b]	5.8[b]	6.1[c]	6.3[b]
GPAB	5.2[c]	5.8[b]	5.2[b]	5.9[c]	6.1[b]
Ogi	6.7[b]	7.4[a]	7.5[a]	7.3[b]	7.1[b]
Cerelac	8.3[a]	7.7[a]	8.3[a]	8.6[a]	8.5[a]
Range	5.0-8.3	5.8-7.7	5.2-8.3	5.8-7.3	6.1-8.5

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

Selection Criteria for Determining Optimal Weaning Food

A ranking system using six nutritional criteria, i.e., protein content, energy value, calcium:phosphorous ratio, total essential 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 9). 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. The GPB had the lowest ranking score followed by GPAB and GPA respectively. Therefore, the GPB sample was concluded to possess the most desirable nutritional profile among the formulated food samples.

Table 9 Ranking of formulated complementary foods to determine optimal nutritional profile

Parameters	Protein (g/100g)	Energy (kcal.)	Ca/P ratio	TEAA	BV	Sensory attributes	Total score
GPA	3	2	2	3	3	2	15
GPB	2	1	1	1	2	1	08
GPAB	1	3	3	2	1	3	13

TEAA = Total essential amino acids; BV = Biological values.

CONCLUSION

The study investigated the comparison of proximate composition, amino acid profile, sensory attributes and nutritional quality of three formulated complementary foods from the combinations of germinated popcorn, bambara groundnut and African locust bean flour. The germinated popcorn-bambara groundnut blends (GPB) was ranked best when compared with other formulated food samples, i.e. GPA and GPAB. 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 the cerelac (a commercial complementary food) in terms of proximate composition.

REFERENCES

ACHI, OK. 2005. The upgrading of traditional fermented foods through biotechnology. In African Journal of Biotechnology, vol. 4, 2005, p. 375-380.

ALOZIE YE. – IYAM, MA. - LAWAL O. – UDOFIA, U. - ANI, IF. 2009. Utilization of Bambara ground flour blends in bread production. In Journal of Food Technology. 7(4), 2009, p.111-114.

ALSMEYER, RH. - CUNNINGHAM, AE. - HAPPICH, ML. 1974. Equations predict PER from amino acid analysis. In Food Technology. 28, 1974, p. 34-40.

ANIGO, KM. – AMEH, DA. – IBRAHIM, S. – DANBAUCHI, SS. 2009. Infant feeding practices and nutritional status of children in North Western Nigeria. In Asian Journal Clinical Nutrition vol. 1, 2009, no.1, p. 12-22.

ANIGO, KM. – AMEH, DA. – IBRAHIM, S. – DANBAUCHI, SS. 2007. Microbiological analyses of commonly used local complementary foods in North Western Nigeria. In Journal Medical Sciences. 7, 2007, no. 5, p. 875-879.

AOAC. (Association of Official Analytical Chemists) (2005). Official Methods of Analysis of the Association of Analytical Chemists International, 18th ed. Gathersburg, MD U.S.A Official methods, 2005.08

AYKROYD, WR. 1981. Legumes in human nutrition. 2nd Edn. FAO. Nutrition Studies. No. 19. Rome, 1981.

BAU, HM. - VILLAUME, CH. - ME´JEAN, L. 2000. Effects of soybean (Glycine max) germination on biologically active components, nutritional values of seeds, and biological characteristics in rats. In Nahrung-Food, 44, 2000, p. 2–6.

BEUCHAT, LR. 1977. Functional and electrophoretic characteristics of succinylated peanut flour protein. In Journal of Agricultural and Food Chemistry, 25, 1977, p. 258–261.

BHATTACHARYA, J. – CURRIE, J. – HAIDER, S. 2004. Poverty, food insecurity, and nutritional outcomes in children and adults. In Journal Health Economic. 23, 2004, p. 839–862.

BLACK, RE. – MORRIS, SS. – BRYCE, J. (2003). Where and why are 10 million children dying every year? In Lancet, vol. 361, 2003, p. 2226-34.

BINDRA GS. - GIBSON RS. - THOMPSON LU. (1986). [Phytate]/[calciumj/[zinc] ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. In Nutrition Research. 6, 1986, p. 475-483.

BROWN, HH. - DEWEY, KG. - ALLEN, LH. 1998. Complementary feeding of young children in developing countries: a review of current scientific knowledge. World Health Organization, Geneva, WHO/NUT/98 (1998).

BRUYERON, O. – DENIZEAU, M. – BERGER, J. - TRÈCHE S. 2010. Marketing complementary foods and supplements in Burkina Faso, Madagascar, and Vietnam: Lessons learned from the Nutridev program. In Food Nutrition Bulletin. 31, 2010, no. 2, p. S154-S167.

BURNS, R. 1971. Method for estimation of tannin in grain sorghum. In Agronomy Journal 63: , 1997, p. 511-512.

CAPPUCCIO, FP. – MCGREGOR, GA. 1991. Does potassium supplementation lower blood pressure? A meta-analysis of published trials. In Journal of Hypertensive. *9:* 1991, p. *465-473.*

COFFMAN, CW. – GARCIA, VV. 1977. Functional Properties and Amino Acid content of a Protein isolate from Mug. Bean Flour. Journal of Food Technology. vol **12, 1977, p** 473-484.

DAELMANS, B. SAADEH, R. 2003. Global initiatives to improve complementary feeding. In: Moreira (eds) Meeting the challenge to improve complementary feeding. SCN News United Nations System Standing Committee on Nutrition, Lavenhem Press, UK: 2003, p. 10-17.

DAVIDSON, S. – PASSMORE, S. – BROCK, JF. – TRUSWELL, AS. 1980. Human Nutrition and Dietetics. Churchill Livingstone, New York. 1980, p. 213-215. www.gastrohepcom/../Arias-3e_chapter_16_Energy_Metabolism pdf.

DAVIES, NT. – CARSWELL, AJP. – MILLS, CF. 1985.The effects ofvariation in dietary calcium intake on the phytate-zinc interaction in rats. In:Mills CF, Bremner I, Chesters JK, eds. Trace elements in man and animals-TEMA 5. Farnham Royal, UK: Commonwealth Agricultural Bureau, 1985, p. 125(abstr).

DEVLIN, MT. 1997. Textbook of biochemistry with Clincal Correlations. 4th ed. Wiley-Liss Inc. New York/Chichester/Toronto. 1997, p. 540-548.

DOP, MC. - BENBOUZID, D. 1999. Regional features of complementary feeding in Africa and the Middle East. *In* : Dop, M.C., Benbouzid, D., Trèche, S., de Benoist, B., Verster, A. & Delpeuch, F. eds. Complementary feeding of young children in Africa and the Middle-East. Geneva: World Health Organization (WHO/NHD/99.3 edt. WHO/AFRO/NUT/99.4): 1999, p. 43-58.

DUNCAN, JG. - BROOKS-GUNN, J. – KLEBANOV, PK. 1994. "Economic Deprivation and Early Childhood Development." In Child Development vol. 65, 1994, no. 2, p. 296-318.

EKA, BE. – ABBEY, BW. – AKANINWOR, JO. 2010. Nutritional Evaluation of Some Traditional Weaning Foods from Akwa Ibom State, Nigeria. In Nigerian Journal of Biochemistry and Molecular Biology, vol.25, 2010, no. 1, p. 65-72.

FAO. 2001. Targeting for nutrition resources for advancing well being. Rome: Food and Agricultural Organization, UN. Geneva. www.fao.org/DOCREP/004/Y1329E/y1329e00.htm. (2001).

FAO. 2004. Undernourishment around the world. In: *The state of food insecurity in the world 2004*. Rome: The Organization, 2004..

FAO/WHO. 1991. CODEX CAC/GL 08, 1991. Codex Alimentarius:Guidelines on Formulated Supplementary Foods for Older Infants and Young Children. Vol. 4, FAO/WHO Joint Publications, 1991, p.144.

FAO/WHO. 1991. Protein quality evaluation. Report of Joint FAO/WHO Expert Consultation. FAO Food and Nutrition paper 51. FAO/WHO. Rome, Italy, 1991, p.1-66

FERNANDEZ, DE. - VANDERJAGT DJ. – WILLIAMS, M. – HUARG, YS. - LUT-TE, C. - MILLSON, M. – ANDREW, R. – PASTUSZYN, A. – GLEW, RH. 2002. Fatty acids, amino acids, and trace mineral analyses of five weaning foods from Jos, Nigeria. In Plant foods for Human Nutrition., 57, 2002, p. 257-274.

FERREIRA, RB. - MELO, TS. – TEIXEIRA, AN. 1995. Catabolism of the seed storage proteins from Lupinus albus: fate of globulins during germination and seedling growth. In Australian Journal of Plant Physiology, 22, 1995, p. 373–381.

FMOH. 2005. National policy on infant and young child feeding in Nigeria, 2005, p. 1-25

GIAMI, SY. - BEKEHAM, DA. 1992. Proximate composition and functional properties of raw and processed full fat fluted pumpkin (*Telferia occidentalis*) seed flour. In Journal of Food Science and Agriculture, **59,** 1992, p.32.

GIBSON, RS. 2006. Zn: the missing link in combating micronutrient malnutrition in developing countries. In Proceeding of Nutrition Society, vol. 65, 2006, p. 51-60.

GIBSON, RS. - SMIT-VADERKOOY, PD. – THOMPSON, L. 1991. Dietary phytate x Ca/Zn millimolar ratios and Zn nutriture in some Ontario preschool children, In Biological. Trace Element Research, vol. 30, 1991, p. 87-94.

GOPALDAS, T. - JOHN C. 1991. Evaluation of a controlled 6 months feeding trial on intake by infants and toddlers fed highenergy-low bulk gruel versus a high energy-high bulk gruel in addition to their habitual home diet. In Journal Tropical Paediatrics, vol. **38**, 1991, p. 278–83.

GRIFFITH, LD. - CASTELL-PEREZ ME. – GRIFFITH, ME. 1998. Effects of Blend and Processing Method on the Nutritional Quality of Weaning Foods Made from Select Cereals and Legumes. In Cereal Chemistry. Vol.75, 1998, no. 1, p.105–112

HALLBERG, RL, - SKANBERG AB. 1987. Phytates and the inhibitory effect of bran on iron-absorption in man. In American Journal of Clinical Nutrition, vol. 45, 1987, no 5, p. 988-996.

HAMBIDGE, KM. – KREBS, NF. – WALRAVENS, PA. 1985. Growth velocity of young children receiving a dietary zinc supplement. In Nutrition Research, vol. I 1985, p.306-l6.

HARIHARAN, M. – NAGA, S. – VANNOORD, T. 1993. Systemic approach to the development of plasma amino acid analysis by high performance liquid chromatography with ultraviolet detection with pre-column derivatization using phenylisothiocynate. In Journal of Chromatography, vol. 621, 1993, p.15-22

HSU, HW. - SUTTON, NE. - BANJO, MO. - SATTERLEE, LD. - Kendrick, JG. 1978. The C-PER and T-PER assays for protein quality. In Food Technology, vol. 32, 1978, no. 68, p.69-73.

IBRAHIM, S. S. – HABIBA, R. A. - SHATTA, A. A. - EMBABY, H. E. (2002). Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. Nahrung/Food, 46, 92–95.

IJAROTIMI OS. - KESHINRO, OO. 2011. Determination of Amino Acid, Fatty Acid, Mineral, Functional and Choking Properties of Germinated and Fermented Popcorn (*Zea mays everta*) Flour. In European Journal of Food Research & Review, vol. 1, 2011, no. 2, p. 102-122.

JACHMANIAN, I. - PERIFANOVA-NEMSKA, M. - GROMPONE, MA. - MUKHERJEE, KD. 1995. Germinating rapeseed as biocatalyst: hydrolysis of exogenous and endogenous triacylglycerols. In Journal of Agricultural and Food Chemistry, vol. 43, 1995, p. 2992–2996.

JANSEN, GR. 1992. Centrally processed weaning foods for use in developing countries. In Food Reviews International, vol. 8, 1992, p. 307–346.

KAKADE, ML. – SIMONS, N. – LEINER, IE. 1969. An evaluation of natural vs. synthetic substrates for measuring anti tryptic activity of soya bean samples. In Cereal Chemistry, vol. 46, 1969, p. 518-526

KING, J. – ASHWORTH, A. 1987. Changes in infant feeding practices in Nigeria: an historical review. Occasional Paper No. 9. London: Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, 1987.

KONO, K. – YOSHIDA, Y. 1989. Current aspect of trace element research in environmental health. In Bulletin of Osaka Medical College, vol.35, 1989, no. (1-2), p. 1-9.

KRETCHMER, N. – BEARD, JL. – CARLSON, S. 1996. "The Role of Nutrition in the Development of Normal Cognition." In American Journal of Clinical Nutrition, vol. 63, 1996, p. 997S-1001S.

LABUDA, J. – KACEROVSKÝ, O. – KOVÁČ, M. – ŠTĚRBA, A. 1982. Výživa a krmenie hospodárskych zvierat. Príroda, Bratislava, 1982, p. 164 s.

LANGFORD, HG. 1983. Dietary potassium and hypertension: epidemiologic data. In Annal. International. Medicine, vol. 98, 1983, no.2, p.770-772

LEACH, HW. - MCCOWEN, LD. - SCHOCH, TJ. 1959. Structure of the starch granules. In: Swelling and solubility patterns of various starches. In Cereal Chemistry, vol. 36, 1959, p. 534-544.

MARERO, LM. - PAJUMO, EM. – LIBRANDO, EC. 1988. Technology of weaning food formulation prepared from germinated cereals and legumes. In Journal of Food Science, vol. **53**, 1988, p.1391 – 1395.

MARTIN, EA. - COOLIDGE, AA. 1978. Nutrition in Action, 4th ed. Holt, R and Wilson Co. New York, USA, 1978.

MBITHI-MWIKYA, S., - VAN CAMP, J. - RODRI´GUEZ, R. - HUYGHEBAERT, A. 2001. Effects of sprouting on nutrient and antinutrient composition of kidney beans. In European Food Research and Technology, vol. 212, 2001, p. 188–191.

MENSAH, P. – TOMKINS, A. 2003. Household-level technologies to improve the availability and preparation of adequate and safe complementary foods. In *Food and Nutrition Bulletin*, vol. 24, 2003, no. 1, p.104–125

MOHAMED, LB. – HUIMING, Z. 2007. Formulation and Nutritional quality of extruded weaning food supplemented with whole egg power. In American Journal of Food Technology, vol. 2, 2007 , no. 6, p. 477-489.

MOHAMED, ME. - AMRO BH. – MASHIER, AS. – ELFADIL, EB. 2007. Effect of Processing Followed by Fermentation on Antinutritional Factors Content of Pearl Millet [Pennisetum glaucum L.] Cultivars. In Pakistan Journal of Nutrition, vol. 6, 2007, no. 5, p. 463-467.

MORRIS, ER. – ELLIS, R. 1985. Bioavailability of dietary calcium-effect of phytate on adult men consuming non-vegetarian diets. In: ACS Symposium Series 75:Nutritional Bioavailability of Calcium. Kies C (eds), p. 63. American Chemical Society: Washington, DC, USA, 1985.

MORRIS, ER. – ELLIS, R. 1989. Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans. In Biological Trace Element Research, vol. 19, 1989, p. 107-117.

MOSHA, AC. - LORRI, WSM. 1987. High-nutrient-density weaning foods from germinated cereals.In: Alnwick D, Moses S, Schmidt OG, eds. Improving young child feeding in Eastern and Southern Africa. Nairobi, New York, Stockholm: IDRC, UNICEF, SIDA, 1987, p. 288–99.

MUHIMBULA, HS. - ISSA-ZACHARIA, A. – KINABO, J. 2011 Formulation and sensory evaluation of complementary foods from local, cheap and readily available cereals and legumes in Iringa, Tanzania. In African Journal of Food Science , vol. 5, 2011 , no. 1, p. 26 – 31.

MÜLLER, O. – GARENNE, M. – KOUYATÉ, B. – BECHER, H. 2003 The association between protein – energy malnutrition, malaria morbidity and all-cause mortality in West African children. In Tropical Med Int Health, vol. 8, 2003, p.507-11.

MUNE-MUNE, MA. - MINKA, SR. - MBOME, IL. - ETOA, FX. 2011. Nutritional Potential of Bambara Bean Protein Concentrate. Pakistan Journal of Nutrition, vol. 10, 2011, no. 2, p. 112-119.

NEMER, L. – GELBAND, H. – JHA, P. 2001. Commission on Macroeconomics and Health. The evidence base for interventions to reduce malnutrition in children under five and school-age children in low- and middle-income countries. CMH working paper no WG5:11. Geneva: World Health Organization, 2001.

NIEMAN, DC. – BUTTERWORTH, DE. – NIEMAN, CN. 1992. Nutrition. WmC. Brown, Dbugye, USA. 1992, p.237-312

NNAM, NM. 2001. Comparison of the protein nutritional value of food blends based on sorghum, bambara groundnut and sweet potatoes. In Infcrnufional Journal of Food Sciences und Nufrifion,*vol. 52,* 2001, p. 25-29.

NNANNA, IA. - PHILLIPS, RD. - MCWATTERS, KH. - HUNG, YC. 1990. Effect of germination on the physical, chemical and sensory characteristics of cowpea products: flour, paste, and akara. In Journal of Agricultural and Food Chemistry, vol. 38, 1990, p. 812–816.

NPC/ICFM. 2009. National Population Commission (NPC) [Nigeria] and ICF Macro. Nigeria Demographic and Health Survey *2008.* Abuja, Nigeria: National Population Commission and ICF Macro, (2009).

OBOH, HA. – MUZQUIZ, M. – BURBANO, C. – CUADRADO, C, - PEDROSA, MM. – AYET, G. – OSAGIE, AU. 2000. Effect of soaking, cooking and germination on the oligosaccharide content of selected Nigerian legume seeds. In Plant Foods for Human Nutrition, vol. 55, 2000, p. 97-110.

OKAKA, JC. - POTTER, NN. 1979. Physicochemical and functional properties of cowpea powders processed to reduce beany flavour. In Journal of Food Science, vol. 44, 1979, p. 1235-1240.

OKOYE, ZSC. 1992. Biochemical Aspects of Nutrition Prentice-Hall of India, New Delhi, 1992, p. 147-105.

OMUETI, O. – OTEGBAYO, B. - JAIYEOLA, O. - AFOLABI, O. 2009. Functional properties of complementary diets developed from soybean (*Glycine max),* groundnut (*Arachis hypogea*) and crayfish (*Macrobrachium SPP*). In EJEAFChe, vol. 8, 2009, no. 8, p.563-573.

OSER, BL. 1959. An integrated essential amino acid index for predicting the biological value of proteins. In A.A. Albanese (Ed.), Protein and amino acid nutrition. New York: Academic Press. 1959, p. 295-311.

OSUNDAHUNSI, OF. - AWORH, OC. 2002. A preliminary study on the use of tempeh based formula as a weaning diets in Nigeria. In Plant foods for Human Nutrition, vol. **57**, 2002, p. 365 – 376.

PODESTA, FE. – PLAXTON, WC. 1994. Regulation of cytosolic carbon metabolism in germinating Ricinus communis cotyledons I. Developmental profiles for the activity, concentration, and molecular structure of the pyrophosphate- and ATP-dependent phosphofructokinases, phosphoenolpyruvate carboxylase and pyruvate kinase. In Planta, 194, 1994, p.374–380.

POLLIT, E. 1994. "Poverty and Child Development: Relevance of Research in Developing Countries to the United States." In Child Development, vol. 64, 1978, no. 2, p. 283-295.

PRICE, ML. - SCOYOC, SV. - BUTLER, LG. 1978. A critical evaluation of the vanillin reaction as an essay for tannin in sorghum grain. In Journal of Agricultural and Food Chemistry, vol. 26, 1978, p.1214-1218

SANDBERG, AS. – ANDERSON, H. – CARLESSON, NG. – SANDSTROM, B. 1987. Degradation products of bran phytate formed during digestion in the human small intestine: effects of extrusion cooking on digestibility. In Journal of Nutrition, vol. 117, 1987, p. 2061-2065.

SNOW, JE. 1974. Flexible packaging and food products compatibility in: Chemistry of food packaging. Swalon (M. ed) American Chemical Society, Washington DC, vol. 82, 1974, p. 84.

SOLOMON, M. 2005. Nutritive Value of three potential complementary foods based on cereals and legumes. In African Journal of Food and Nutritional Sciences, vol. 5, 2005, no. 2, p. 1-14.

SYED, AS. – AURANG, Z. – TARIQ, M. – NADIA, N. – SAYED, JA. – MUHAMMAD, S. - ABDUL, A. – ASIM, M. 2011. Effects of sprouting time on biochemical and nutritional qualities of Mungbean varieties. In African Journal of Agricultural Research, vol. 6, 2011, no. 22, p.5091-5098.

TEMPLE, VJ. – BADAMOSI, EJ. – LADEJI, O. – SOLOMON, M. 1996. Proximate chemical composition of three locally formulated complementary foods. In West African. Journal of Biological Sciences, vol. 5, 1996, p. 134-143.

TRAORÉ, T. 2005. Development and evaluation of a strategy for the improvement of the complementary food of the young children in Burkina Faso, Ph.D.Thesis, University of Ouagadougou, Burkina Faso, (2005).

TURNLUND, JR. – KING, JC. – KEYES, WR. – GONG, B. – MICHEL, MC. 1984. A stable isotope study of zinc absorption in young men: effects of phytate and α-cellulose. In American Clinical Nutrition, vol. 40, 1984, p. 1071-1077.

UWAEGBUTE, AC. - IROEGBU, CU. – EKE, O. 2000. Chemical and sensory evaluation of germinated cowpeas (Vigna unguiculayta) and their products. In Food Chemistry, 68, 2000, p. 141–146.

WAKIL, SM. - ONILUDE, AA. 2009. Microbiological and chemical changes during production of malted and fermented Cereal-Legume weaning foods. In Advances in food sciences. vol. 31, 2009, no. 3, p. 139-145.

WHEELER, EI. – Ferrel, RE. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chemistry, 48, 1971, p. 312-320.

WHO. 2002. Childhood nutrition and progress in implementing the International Code of Marketing of Breast-milk Substitutes. Geneva, World Health Organization, 2002 (document A55/14).

ZIEGLER P. 1995. Carbohydrate degradation during germination. In J. Kigel & G. Galili (Eds.), Seed development and germination. New York: Marcel Dekker, Inc. 1995, p.447–474.