NUTRIENT COMPOSITION AND BIO-NUTRITIONAL CHARACTERISTICS OF POTENTIAL COMPLEMENTARY FOODS PRODUCED FROM POPCORN, SOYBEAN CAKE AND WONDERFUL KOLA FLOUR

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Abstract
Food materials (popcorn, soybean and wonderful kola seeds) were processed into flour, and blended to obtain three food samples, that is, raw (RPW), blanched (BPW) and fermented (FPW) flour mixed. The blended samples were evaluated for chemical and biological properties. The range values of crude protein and energy of complementary foods (CF) were 13.84±0.79 – 19.16±0.95 g/100g and 427.15±2.32 - 430.85±1.95 kcal/100g respectively. The protein content of experimental food samples were higher than control (BTF) (14.84±0.02 g/100g), except BPW, whereas, energy values were significantly ($p<0.05$) lower than BTF. Potassium and zinc were the highest and lowest mineral element in the food samples respectively. The essential amino acid composition of the food samples ranged from 31.56±0.01 in RPW to 33.50±0.20 mg/100g in BPW, and these values were significantly higher than PCN (18.37±0.03) and BFT (23.66±0.11 mg/100g). The biological value, protein efficiency ratio and protein rating of experimental food samples ranged from 69.45% in RPW to 78.41% in FPW, 0.43 in BPW to 0.49 in FPW and 0.91in BPW to 1.20 in FPW respectively, and these values were significantly ($p<0.05$) lower than in BTF, but higher than in PCN. The rats fed on experimental food samples had better growth performance than those fed on PCN, but lesser than those fed on BTF. The hematological variables, that is, red blood cell (RBC), pack cell volume (PCV), heamoglobin (Hb) and lymphocytes concentrations of the rats fed on experimental complementary foods ranged as follows: 9.78 - 14.59 x 10^6 mm^3, 38.0 - 47.2%, 12.7 - 15.9 g/dl and 62 - 63% respectively, and were within the normal range values. The present study reported on nutrient compositions, in vivo protein quality and growth performance of formulated complementary foods from the combination of popcorn, soybean cake and wonderful kola flour. The FPW sample had the best in terms of nutrient compositions and growth performance in rats, hence the diet is suitable for complementary food and also for the treatment of children already suffering from protein-energy malnutrition.

Keywords: complementary foods, amino acid profile, bio-efficacy characteristics

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1. INTRODUCTION

Child malnutrition is a major public health problem in many parts of developing countries, and it is the main underlying causes of death among children in these communities (FAO, 2008). Poor nutritional quality, inadequate complementary food intakes and improper feeding practices have been identified as among the major causes of child malnutrition in developing countries (Rice *et al*., 2000; Müller *et al.,* 2003). It is well established that protein-energy malnutrition has negative effects on the child growth (Umeta *et al*., 2000), cognitive development (Hamadani *et al*., 2001; Berkman *et al*., 2002), and it increases infant morbidity (Kalanda *et al*., 2006) and mortality rate (Edmond *et al*., 2006). For these reasons, adequate complementary food is necessary for optimum growth and development of infants (WHO, 2001).

Finding has shown that as the baby grows and becomes more active, breast-milk alone is no longer sufficient to meet his or her nutritional and physiological needs (Ruel *et al*., 2003). Hence, introduction of qualitative complementary foods become necessary. In developing countries, traditional complementary foods are mainly from local staple foods such as cereals and tubers, which are with low protein-density and bulky (Ukegbu and Anyika, 2012). These traditional complementary foods are often failed to meet the nutritional needs of the infants, due to lack of adequate nutrients and poor complementation of locally available food materials (Ukegbu and Anyika, 2012). In an attempt to improve the nutritive values of local

complementary foods, various forms of economical protein–rich plant combinations are being used in Nigeria and other developing countries to reduce the prevalence of chronic and acute malnutrition among children less than 5 years (Obatolu and Cole, 2003; Wadud *et al*., 2004; Onilude *et al*., 2004; Ikujenlola and Fashakin, 2005; Ijarotimi and Ayantokun, 2006). In Nigeria, quite a number of complementary foods from locally available food materials like cereals and legumes have been formulated, but no work has been carried out on the formulation of complementary foods from the combinations of popcorn, soybean and wonderful kola. Popcorn is a cereal with moderate protein, and like other cereal grains; it is deficient in lysine and tryptophan. Soybean is a legume and high in protein compared to other legumes, while wonderful kola contains essential nutrients and bioactive compounds to boost immune system. The present study, therefore, aimed at formulating complementary foods from these locally available food materials that could be nutritionally meet infant requirements and boost their health status.

2. MATERIALS AND METHODS

Sources of food materials and Wistar rats

Freshly harvested *Buchholzia coriacea* seeds were bought from Ojee market in Ibadan, popcorn kernels and groundnut oil were purchased from Erekensan market, Akure, while soycake was obtained from JOF Limited, Owo, Ondo State, Nigeria. The BP-100 Therapeutical foods (BTF) produced by UNICEF was obtained from nutrition rehabilitative unit, Health center, Ado-Ekiti. The Wistar albino rats were purchased from Central Animal House, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Statement of Animal Rights

The study protocol was approved by the Ethical Committee for Laboratory Animals of School of Agriculture and Agricultural Technology, Akure, Nigeria (FUTA/SAAT/2017/033). The experiments on animals were conducted in accordance with the force laws and regulations as regards care and use of laboratory animals.

Processing of food materials into flour

Popcorn flour: Raw popcorn kernels were sorted, winnowed, manually washed with distilled water and drained. The drained popcorn kernels were blanched at 100 ºC for 40 min. to enhance softening of the kernels prior to the next processing stage. The kernels were oven dried at 60 ^{o}C (Plus11 Sanyo Gallenkamp PLC, UK) for 20 h, milled using Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard) to obtain popcorn flour. The flour was packed in a plastic container, sealed and stored at room temperature (~27 ^{o}C) until required for use.

Wonderful kola (B. coriacea) flour: The fresh wonderful kola was cleaned by the double disinfection method. They were washed thoroughly with distilled water to remove adhering particles after which they were soaked in 80% ethanol for 30 min. The seeds were rinsed with distilled water and then washed with aqueous sodium hypochlorite (NaClO) to reduce surface contamination and rinsed again with distilled water. The wonderful kola seeds were divided into three parts, one of the parts was blanched and the second parts was blanched and fermented for three days using local method. The raw, blanched and fermented seeds were oven dried at 60 ^{o}C (Plus11 Sanyo Gallenkamp PLC, UK) for 8 h, milled using Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard) to obtain raw, blanched and fermented wonderful kola seed flour. The flour was packed in a plastic container, sealed and stored at room temperature (~27 ^{o}C) until required for use.

***Soybean cake flour*:** Defatted soybean cake was further cleaned, oven dried at 60 ^{o}C (Plus11 Sanyo Gallenkamp PLC, UK) for 5 h, milled using Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard) to obtain soycake flour. The flour was packed in a plastic

container, sealed and stored at room temperature (~27 ^{o}C) until required for use.

Formulations of food samples

The popcorn, defatted soybean cake and wonderful kolanut flour were mixed in different proportions using NutriSurvey Linear Programming software version 2007 with reference to protein requirements of children to obtain three formulations as follows:

Table 1. Proportion of popcorn, soybean, *Groundnut oil* and *Buccholzia coriacea* seeds samples in the formulated food samples

Samples	Popcorn	Soybean cake	*Groundnut oil*	*Buccholzia coriacea* seeds
RPW	60	20	10	10
BPW	60	20	10	10
FPW	60	20	10	10

RPW = popcorn, soycake and raw wonderful kola flour, BPW = popcorn, soycake and blanched wonderful kola flour, FPW = popcorn, soycake and fermented wonderful kola flour.

Chemical analyses of complementary foods produced from popcorn, soybean cake and wonderful kola flour blends

Proximate compositions determination

Proximate compositions of the formulated complementary foods were determined using the methods of Association of Official Analytical Chemists (AOAC) (2012). Moisture content was determined in a hot-air circulating oven (Galenkamp). Ash was determined by incineration (550 °C) of known weights of the samples in a muffle furnace (Hotbox oven, Gallencamp, UK, size 3) (AOAC, 2012). Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40 to 60 °C) using TecatorSoxtec (Model 2043(20430001), 69, Slandegarupgade, DK-3400, Hilleroed, Denmark) (AOAC, 2012). Protein content (N × 6.25) was determined by the micro-Kjeldahl method (Method No 978.04) (AOAC, 2012). Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (AOAC, 2012). The available carbohydrate content was determined by difference, that is, addition of all the percentages of moisture, fat, crude protein, ash and crude fibre and 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 energy value of the samples were estimated (in kcal/g) by multiplying the percentages of crude protein, crude lipid and carbohydrate with the recommended factors 4.0, 9.0 and 4.0 respectively as proposed by Iombor *et al*. (2009).

Mineral compositions determination

Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn) were determined using Atomic Absorption Spectrophotometer (AAS Model SP9). Sodium (Na) and potassium (K) were determined using flame emission photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) with NaCl and KCl as the standards (AOAC 2012). Phosphorus was determined using Vanodo-molybdate method.

Determination of amino acid compositions of complementary foods produced from popcorn, soybean cake and wonderful kola flour blends

Amino acids profile of formulated complementary foods using the method described by AOAC (2012). The complementary food samples were digested using 6N HCl for 24 h. Amino acids were determined using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter Inc., Fullerton, Calif., USA) employing sodium citrate buffers as step gradients with the cation exchange post-column ninhydrin derivatization method. 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. The cysteine and methionine contents were determined after performic acid oxidation and tryptophan content was measured colorimetrically (Pinte´r-Szaka´cs and Molna´r-Perl, 1990).

Determination of antinutritional Factors of complementary foods produced from popcorn, soybean cake and wonderful kola flour blends

Determination of phytate: The method of AOAC (2005) was employed for phytin determination. Each sample (2.0 g) was dissolved in 100 ml of 2% HCl (v/v) for 3 h and filtered. The filtrate (25 ml) was placed in a 100 ml conical flask and 5 ml of 0.03% NH_4SCN solution was added as indicator, while 50 ml of distilled water was added. This was titrated against ferric chloride solution which contained 0.005 mg of Fe^{3+} per ml of $FeCl_3$. The equivalent was obtained, while the phytate content in mg/100g was calculated.

Iron equivalent = titre value x 1.95 x 1.19 = Phytin P mg/g

Phytic acid = titre value x 1.95 x 1.19 x 3.5 mg/phytic acid

$$\% \text{ Phytic acid} = \frac{V \times 8.24}{1000} \times \frac{100}{\text{Weight of sample}}$$

Where V = titre (ml)
Digestible phosphorus = total phosphorus – phytin P

***Determination of tannin*:** The tannin content of the seed flours was determined by modifying the procedure of Jaffe (2003). Finely ground sample (0.2 g) was weighed with a 50 ml sample bottle; about 10ml of 70% aqueous acetone was added and covered properly. The bottles were put in an ice bath shaker and shaken continuously for 2 h at 30 ^oC. Each solution was then centrifuged at 3000 xg and the supernatant stored in ice, 0.2 ml of each supernatant was pipetted into test tubes and 0.8 ml of distilled water was added. Standard tannic acid solution was prepared from a 0.5 mg/ml stock solution and the solution was made up to 1 mL with distilled water. Folin ciocalteau reagent (0.5 mL) was added to both the sample and standard, followed by 2.5 ml of 20% Na_2CO_3 solution. The solutions were then incubated for 40 min at room temperature after which their absorbance was read at 725 nm against a reagent blank. The concentration of the sample was obtained from a standard tannic acid curve.

***Determination of oxalate*:** Oxalate was determined according to the method of Munro (2000). One gram of each sample was weighed into 100 ml conical flask, 75 mL of 3M H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1hr and then filtered through Whatman No 1 filter paper. Exactly 25 ml of the filtrate was collected and titrated hot (80 - 90 ^oC) against 0.1M $KMnO_4$ solution to the point when a faint pink colour appeared and persisted for at least 30 s.

$$\text{Oxalate (mg/g)} = V_T \times 0.9004$$

Where, V_T = Titre volume (ml).

***Determination of saponin*:** The method described by Obadoni and Ochuko (2001) was used. Each of the sample (20 g) was put into a conical flask and 100 mL of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55 ^oC. The mixture was filtered and the re-extracted with another 200 ml of 20% ethanol. The combined extracts were evaporated to remain 40 mL over water

bath at about 90 °C. The concentrate was transferred into 250 mL separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the other was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 ml of 5% aqueous sodium chloride solution. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was then calculated as percentage weight of the sample.

$$\% \text{ Saponin} = \frac{\textit{Weight of saponin extract}}{\text{Weight of sample}} \text{x} 100$$

***Determination of flavonoid*:** Each sample (10 g) was extracted with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and dried to a constant weight (Boham and Kocipal-Abyazan, 1994).

$$\% \text{ Flavonoid} = \frac{\textit{Weight of extracted powder}}{\text{Weight of sample}} \text{x } 100$$

***Determination of alkaloid*:** Each sample (5 g) was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hr. This was filtered and the extract was concentrated on water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was completed. The whole suspension was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue which is the alkaloid was dried and weighed to determine the percentage composition (Harbone, 1994).

$$\% \text{ Alkaloid} = \frac{\textit{Weight of extracted powder}}{\text{Weight of sample}} \text{x } 100$$

***Determination of Trypsin Inhibition Activity (TIA)*:** 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 (Griffiths, 2000). The sample (1 g) was extracted continuously at ambient temperature for 3 h with 50 ml, 10 mM NaOH using a mechanical shaker (GallenKamp orbital shaker Surrey, UK). The resulting slurry was adjusted to pH 9.4 - 9.6 using1M NaOH. After extraction, the suspension was shaken and diluted with distilled water such that 1 ml of the extract produced trypsin inhibition of 40-60% at 37 °C. The respective dilutions was noted. Consequently, TIA was calculated in terms of mg pure trypsin (Sigma type lll, lot 20H0868)

$$\text{TIA} = \frac{2.632\text{DA mg pure trypsin inhibited g} - 1\text{ g sample}}{\text{Weight of Sample}} \text{x } 100$$

Where D is the dilution factor, A is the change in absorbance at 410mm due to trypsin inhibition per ml diluted sample extract

***Determination of total phenolic*:** Total phenolic content was determined using Folin–Ciocalteu (George´ *et al*., 2005) with minor modifications, using gallic acid (Sigma, St. Louis, USA) as standard. The sample (5 mg) was dissolved in 5 ml of a methanol:water mixture (50:50 v/v). The solution containing the sample was added to a series of tubes and the volume was made up to 100 ml with the methanol: water mixture (50:50 v/v). Five hundred microlitres of 50% Folin–Ciocalteu reagent was added to each tube and mixed. The mixture was then allowed to stand for 10 min followed by the addition of 1.0 mL of 20% sodium bicarbonate. After 10 min incubation at ambient temperature, the mixture was centrifuged at 10,000 xg in Kubota 6800 (Kubota Co., Osaka, Japan) for 5 min and the absorbance of the supernatant was measured at 700 nm. The total phenolics content was expressed as gallic acid equivalents (GAE) in mg per gram of dry sample.

Nutritional evaluation of complementary foods produced from popcorn, soybean cake and wonderful kola flour blends in rats

***Animal experimental design*:** Experimental animals: Forty-two male and female weanling Wistar Albino rats of 4 weeks old were purchased from Central Animal House, college of Medicine, University of Ibadan, Ibadan, Nigeria. The rats were divided into six groups consisting 7 male and female rats per group, and were housed individually in metabolic cages in a climate-controlled environment with free access to feed and water. The rats were allowed to acclimatize to the new environment for 4 days. After four days of adaptation period, the animals were reweighed. The rats in each group were fed on BP-100 Therapeutical foods (BTF) (a product of UNICEF), local complementary food (Ogi), experimental diets (RPW, BPW, FPW, PCN) and basal diet with water *ad libitum* each day for 28 days.

Anthropometric measurements: Weights of the rats were measured using a digital weighing scale (Salter, SL20348, London, UK) calibrated to the nearest 0.1 kg. The lengths of the rats were measured using a meter board calibrated to the nearest 0.1 cm, and the measurement was done by stretching the animal along the meter board with its nostril touching the zero mark and the measurement was taken between nose and anus distance to the nearest 0.1 cm. The weight-for age, Length-for age and body mass index-for-age (BMI = body mass (g) / length $(cm)^2$) of the rats were calculated to determine the effects of formulated complementary foods on the growth and development of the animals (Novelli *et al*., 2007).

Haematological evaluations: At the end of the feeding trial (28 days), the rats were starved for 12 h overnight and weighed. Before being sacrificed, each rat was anaesthetised with chloroform inside a desiccator. Blood sample was collected via cardiac punctured into sample bottles containing a few milligram of EDTA for haematological analysis. The packed cell volume (PCV) was estimated by spinning about 75µl of each blood sample in heparinised capillary tubes in a haematocrit micro-centrifuge for 5 minutes, and the total red blood cell (RBC) and white blood cell (WBC) counts were determined. The haemoglobin concentration (Hbc) was estimated using cyanomethemoglobin method while Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated (Boyel and Shepiengh, 2001)

Sensory evaluation of complementary foods produced from popcorn, soybean cake and wonderful kola flour blends

Sensory evaluation was performed on the constituted experimental food samples using the descriptive 5-point Hedonic scale rating with 20 panelists that were familiar with the control sample. The rating was, 5 like extremely and 1 dislike extremely for each attribute evaluated.

The assessments were conducted in a well lit room designed for sensory evaluation in the Department of Food Science and Technology. Each of the formulated complementary foods was prepared by stirring flour in boiling water 1:4 (v/v) of flour to water dispersion at 100 ^{o}C for 20 min. Panelists were from the University community and cut across age and sex (Iwe, 2002).

Statistical analysis

The results obtained from the various analyses in triplicates were analysed using SPSS version 16.0. The mean and standard error of means (SEM) of the triplicate analyses were calculated.

The analysis of variance (ANOVA) was performed to determine significant differences between the means, while the means were separated using the New Duncan Multiple Range Test (NDMRT) at $p<0.05$.

3. RESULTS AND DISCUSSION

Nutritional compositions of formulated complementary foods

The proximate composition of formulated complementary foods (FCF) is presented in

Table 2. Moisture contents of the samples ranged from 7.41±0.04 mg/100g in FPW sample to 7.72±0.72 mg/100g in BPW sample. These values were higher when compared to the moisture content of BP-100 therapeutical food (BTF) complementary food (a complementary food formulated by UNICEF for the treatment of severe protein-energy malnutrition). However, the moisture content of the samples were still within the FAO recommended value for flour samples (<10 mg/100g), and this implies that the FCF could be relatively stored for a long period before it starts to deteriorate. The amount of moisture content in flour samples usually determines the activities of micro-organisms, which in turns determine the shelf life of the flour product. Studies have shown that high moisture contents of food products facilitate the activities of micro-organisms, reducing storage period and nutritional qualities of the food products (Olitino *et al*., 2007; Alozie *et al*., 2009; Afolabi, 2014). Crude protein content of FCF varied from 13.84±0.79 g/100g in BPW to 19.16±0.95 g/100g in FPW, and were comparatively higher than BTF (14.84±0.02 g/100g). The protein contents of FCF in the present study were comparable to the reports of Anigo *et al*. (2010) and Solomon (2005). The study further established that the protein content of FPW (a fermented food sample) was significantly ($p<0.05$) higher than RPW and BPW samples. This finding agreed with the report that fermentation usually increased the nutritive values of food products (Kohajdová and Karovičová, 2007). Scientific studies have shown that fermentation have multiple effects on the nutritional value of foods by decreasing the level of carbohydrates as well as some non-digestible poly- and oligosaccharides, synthesized certain amino acids and B group vitamins (Nout and Ngoddy, 1997) and lowers the content of anti-nutrients such as phytates, tanins, polyphenol in food products (Sindhu and Khetarpaul, 2001). Energy values of formulated foods ranged from 427.15±2.32 kcal in RPW to 430.85±1.95 kcal in BPW, and were significantly lower ($p<0.05$) than BTF sample (518.44±0.49 kcal).

Mineral composition of formulated functional foods is presented in Table 3. The mineral compositions of the formulated diets varied as follows: 176.50±0.50 – 224.25±0.25 mg/100g for Phosphorous, 38.75±0.25 – 42.75±3.25 mg/100g calcium, 1.24±0.01 – 1.68±0.02 mg/100g iron, 12.13±0.02 – 96.5±0.50 mg/100g magnesium and 62.45±0.07 – 79.79±0.23mg/100g sodium. For the remaining minerals, the values were 0.12±0.01 – 0.43±0.07, 60.03±0.51 – 70.57±0.53, 6.95±0.05 -11.73±0.57 and 0.40±0.10 - 1.63±0.07 mg/100g for zinc, potassium, copper and manganese respectively. Lead, chromium and selenium were not detected in any of the functional food.

Table 2. Nutrient compositions (g/100g) of experimental food samples

Samples	Moisture (g/100g)	Fibre (g/100g)	Fat (g/100g)	Ash (g/100g)	Protein (g/100g)	CHO (g/100g)	Energy (kcal)
RPW	7.68^{a}	3.39^{c}	10.87^{c}	3.39^{a}	17.90^{b}	64.44^{c}	427.22^{c}
	±0.08	±0.09	±0.25	±0.15	±0.20	±0.24	±1.42
BPW	7.72^{a}	3.84^{b}	11.97^{b}	3.41^{a}	13.84^{c}	66.94^{b}	430.85^{b}
	±0.72	±0.42	±0.71	±0.13	±0.79	±0.44	±1.95
FPW	7.41^{b}	2.79^{d}	10.26d	3.25^{b}	19.16^{a}	64.56^{c}	427.15^{c}
	±0.04	±0.06	±0.41	±0.14	±0.95	±1.30	±2.32
PCN	7.20^{c}	0.85^{e}	5.10^{e}	1.13^{c}	6.67^{d}	86.26^{a}	417.53^{d}
	±0.20	±0.01	±0.13	±0.05	±0.49	±0.30	±0.38
BTF	6.09^{d}	9.85^{a}	34.30^{a}	3.42^{a}	14.84^{c}	37.61^{d}	518.44^{a}
	±0.09	±0.16	±0.25	±0.03	±0.02	±0.42	±0.49
RDA	<10	<5	10-25	10-25	>15	64	400-425

Means (±SEM) with different alphabetical superscripts in the same column are significantly different (P<0.05). *FAO/WHO (1991).

Table 3: Mineral compositions (mg/100g) of experimental food samples

Parameters	RPW	BPW	FPW	PCN	BTF	*RDA (mg/day)
P	176.50^{c}±0.50	192.75^{b}±0.25	224.25^{a}±0.25	86.03^{d}±0.07	54.70^{e}±0.60	1,000
Ca	42.75^{c}±3.25	38.75^{d}±0.25	39.75^{d}±0.55	68.89^{a}±0.24	56.00^{b}±1.00	1,000-1,300
Fe	1.24^{c}±0.01	1.68^{b}±0.02	1.65^{b}±0.05	0.95^{d}±0.15	0.28^{a}±0.015	8 - 18
Mg	25.75^{d}±0.25	96.50^{b}±0.50	12.13^{e}±0.02	35.01^{c}±0.09	74.00^{b}±1.00	400 - 420
Na	79.79^{a}±0.23	71.99^{b}±0.45	62.45^{c}±0.07	14.93^{d}±0.37	6.40^{e}±0.10	460-920
Zn	0.43^{c}±0.07	0.18^{d}±0.02	0.12^{e}±0.01	0.87^{b}±0.06	8.000^{a}±0.10	8 - 14
K	70.57^{b}±0.53	68.96^{b}±0.02	60.03^{c}±0.51	102.96^{a}±0.57	65.45^{e}±0.45	2,800 - 3,800
Cu	6.95^{c}±0.05	11.73^{a}±0.57	10.98^{b}±0.32	1.31^{e}±0.01	3.15^{d}±0.15	1.2 – 1.7
Mn	0.45^{c}±0.05	1.63^{b}±0.07	0.40^{c}±0.10	1.90^{a}±0.03	0.20^{d}±0.10	5 - 5.5
Pb	-	-	-	-	-	-
Cr	-	-	-	-	-	-
Se	-	-	-	-	-	-
Na/K	1.14^{a}±0.02	1.05^{b}±0.03	1.04^{b}±0.03	0.15^{c}±0.02	0.10^{d}±0.01	1.4-3.4
Ca/P	0.24^{c}±0.02	0.20^{c}±0.02	0.18^{c}±0.03	0.80^{b}±0.02	1.03^{a}±0.02	1.6-3.6
Ca/Mg	1.66^{c}±0.00	0.41^{e}±0.00	3.27^{a}±0.01	1.96^{b}±0.02	0.76^{d}±0.03	2-11
Ca/K	0.61^{b}±0.03	0.57^{c}±0.01	0.66^{b}±0.02	0.67^{b}±0.01	0.86^{a}±0.03	2.2-6.2
Na/Mg	3.10^{b}±0.01	0.75^{c}±0.01	5.13^{a}±0.02	0.43^{d}±0.01	0.09^{e}±0.00	2-6
Zn/Cu	0.06^{c}±0.02	0.02^{d}±0.01	0.01^{d}±0.01	0.66^{b}±0.02	2.54^{a}±0.11	2.0-4.0
Fe/Cu	0.18^{c}±0.02	0.15^{c}±0.02	0.15^{c}±0.01	0.30^{a}±0.02	0.21^{b}±0.01	0.2 – 1.6

Means (±SEM) with different alphabetical superscripts in the same column are significantly different (P<0.05).
*FAO/WHO (1991).

The mineral compositions of the blends in this study were comparable to that of BTF, but lower than those of FAO recommended values. The sodium/potassium (Na/K) molar ratios of FCF ranged from 1.04 in FPW to 1.14 in RPW sample, and were comparatively higher than control sample (0.10). However, the values were within FAO/WHO (1991) recommended values (1.4 - 3.4). The Ca/P molar ratios of the formulations ranged between 0.18 and 0.24, and were lower than BTF sample and that of FAO/WHO (1991) recommended value (1.6-3.6). This observation might be due to fortification of BTF with micronutrient during its production, which was not applicable to the food samples in this present study.

The amino acid profile of formulated complementary foods is shown in Table 4. Non-essential amino acid composition showed that glutamic acid and cysteine had the highest and lowest concentration with range values of 12.48±0.01 - 13.19 and 1.15±0.03 to 1.34±0.02 mg/100g protein content respectively. Essential amino acids had leucine (6.28±0.02 and 7.39±0.01 g/100g protein) as the most abundant, while methionine was the lowest concentration in RPW and FPW samples, and tryptophan for BPW sample. The highest value of glutamic acid observed in this study agreed with the reports that glutamic acid is the most abundant amino acid in plant-based food samples (Omoyeni *et al.*, 2015). Comparatively, the amino acid compositions of FCF were significantly ($p<0.05$) higher than that of BTF (a control food sample).

Antinutrient compositions of complementary foods produced from popcorn, soybean cake and wonderful kola flour blends

The antinutrient compositions (mg/g) of formulated complementary foods are presented in Table 5. The concentrations of phytochemical in the formulated functional foods ranged as follows: 5.27±0.18 - 9.27±0.02 mg/g for tannin, 1.19±0.00 - 2.68±0.09 mg/g for polyphenol, 17.30±0.00 - 34.61±0.82mg/g for phytate and 0.73±0.00 - 4.00±0.18mg/g for saponin; while flavonoid, trypsin, alkaloid and oxalate concentrations were 0.97±0.00 - 8.98±0.24 mg/g, 0.40±0.00 – 0.48±0.01, 0.10±0.00 – 4.65±0.00 and 0.28±0.00 - 0.36±0.00 mg/g respectively.

Table 4: Amino acid profile (mg/100g) of experimental food samples

Parameters	RPW	BPW	FPW	PCN	BTF	*RDA (mg/100g b.w)	
						A	C
Non-essential amino acids							
Arginine	5.98b±0.00	6.27^{a}±0.03	5.98^{b}±0.01	4.82^{c}±0.02	4.89^{c}±0.01	-	-
Aspartic	11.06^{b}±0.02	11.28^{a}±0.01	10.70^{c}±0.05	6.14^{e}±0.02	8.08^{d}±0.01	-	-
Serine	3.53^{c}±0.02	4.14^{b}±0.01	2.92^{d}±0.02	4.79^{a}±0.01	3.85^{c}±0.05	-	-
Glutamic	13.18^{b}±0.02	13.19^{b}±0.01	12.48^{c}±0.01	17.61^{a}±0.01	10.40^{d}±0.02	-	-
Proline	3.04^{b}±0.00	3.28^{a}±0.02	3.20^{a}±0.05	2.49^{c}±0.01	2.49^{c}±0.02	-	-
Glycine	4.49^{a}±0.01	4.04^{c}±0.01	3.94^{d}±0.03	4.13^{b}±0.01	3.67^{e}±0.01	-	-
Alanine	4.26^{a}±0.01	3.92^{b}±0.02	3.50^{d}±0.01	3.64^{c}±0.03	3.53^{d}±0.02	-	-
Cystein	1.20^{c}±0.01	1.34^{b}±0.02	1.15^{e}±0.03	1.94^{a}±0.00	0.85^{d}±0.02	-	-
Tyrosine	3.16^{c}±0.00	3.34^{b}±0.02	3.31^{b}±0.01	4.04^{a}±0.01	2.83^{d}±0.01	-	-
Essential amino acids							
Histidine	2.23^{a}±0.02	2.31^{a}±0.01	2.28^{a}±0.02	1.54^{c}±0.04	1.80^{b}±0.01	1.0	-
Lysine	5.28^{b}±0.02	5.40^{a}±0.01	4.93^{c}±0.02	1.73^{e}±0.02	2.69^{d}±0.01	3.0	6.4
Threonine	3.45^{b}±0.01	3.78^{a}±0.02	3.21^{c}±0.01	1.12^{e}±0.01	2.24^{d}±0.01	1.5	3.7
Valine	3.87^{c}±0.01	4.12^{b}±0.00	5.59^{a}±0.02	2.73^{e}±0.05	3.64^{d}±0.26	2.6	3.8
Meth./Cyst.	1.30^{b}±0.01	1.34^{a}±0.02	1.24^{b}±0.02	1.16^{c}±0.03	1.28^{b}±0.01	1.5	2.7
Isoleucine	3.31^{c}±0.01	3.44^{b}±0.00	3.18^{d}±0.01	3.58^{a}±0.01	3.07^{e}±0.01	2.0	3.1
Leucine	6.28^{c}±0.02	7.39^{a}±0.01	6.56^{b}±0.01	3.76^{e}±0.01	4.63^{d}±0.02	3.9	7.3
Phenylal./Tyr	4.69^{a}±0.01	4.35^{b}±0.02	4.27^{c}±0.02	2.06^{e}±0.01	3.33^{d}±0.01	2.5	6.9
Tryptophan	1.31^{a}±0.01	1.30^{a}±0.01	1.31^{a}±0.01	0.85^{c}±0.00	1.01^{b}±0.01	0.4	1.25
∑EAAs	31.56^{c}±0.01	33.50^{a}±0.20	32.45^{b}±0.02	18.37^{e}±0.03	23.66^{d}±0.11		

*Means (±SEM) with different alphabetical superscripts in the same row are significantly different (P<0.05). RDA Sources:*FAO/WHO (1991), *FAO/WHO/UNU (2007). A = Adult,* C= *Children (<5 yrs.)*

Table 5: Phytochemical compositions (mg/g) of experimental food samples

Parameters	RPW	BPW	FPW	Critical values
Tannin	9.27^{a}±0.02	5.27^{c}±0.18	7.57^{b}±0.00	3.0mg/100g
Polyphenol	2.68^{a}±0.09	1.19^{b}±0.00	1.32^{c}±0.02	-
Phytate	34.61^{a}±0.82	17.30^{c}±0.00	20.60^{b}±0.00	5-6g/100g
Saponin	4.00^{a}±0.18	0.73^{c}±0.00	1.91^{b}±0.09	-
Flavonoid	8.98^{a}±0.24	0.97^{b}±0.00	0.97^{b}±0.00	-
Trypsin	0.45^{b}±0.01	0.48^{a}±0.01	0.40^{c}±0.00	0.25g/100g
Alkaloid	0.10^{c}±0.00	2.77^{b}±0.01	4.65^{a}±0.00	-
Oxalate	0.28^{b}±0.00	0.36^{a}±0.00	0.36^{a}±0.00	0.25g/100g

Mean± SEM across the rows with different superscripts differ significantly at P<0.05.

Comparatively, the concentrations of tannin, polyphenol, phytate, saponin and flavonoid in BPW and FPW were significantly lower than in RPW, except for trypsin, alkaloid and oxalate.

This observation could be attributed to the effects of processing methods that were used to prepare the flour samples. Evident has shown that food processing may alter the concentrations or anti-oxidative potentials of phytochemicals (Rungapamestry *et al.*, 2007; Watchtel-Galor *et al.*, 2008). Nutritionally, it is evident that low intakes of phytochemicals offer health benefits than when taken at high dosage (Rui, 2003), and that intakes of phytochemicals at high dosage may increases the risk of toxicity (Rui, 2003; Song *et al.*, 2010; Cox *et al.*, 2010).

The health-promoting effects of many phytochemicals are attributed mainly to their antioxidant activity, which helps to prevent the formation of hydroxyl, superoxide and peroxyl radicals in man, which can lead to age related degenerative conditions, cancer, diabetes and other diet related diseases (Eberhardt *et al.*, 2000; Arab and Steck, 2000).

Growth performance of Wistar rats fed on formulated complementary foods

The growth patterns of albino rats fed with formulated foods and control food samples are presented in Figures1.

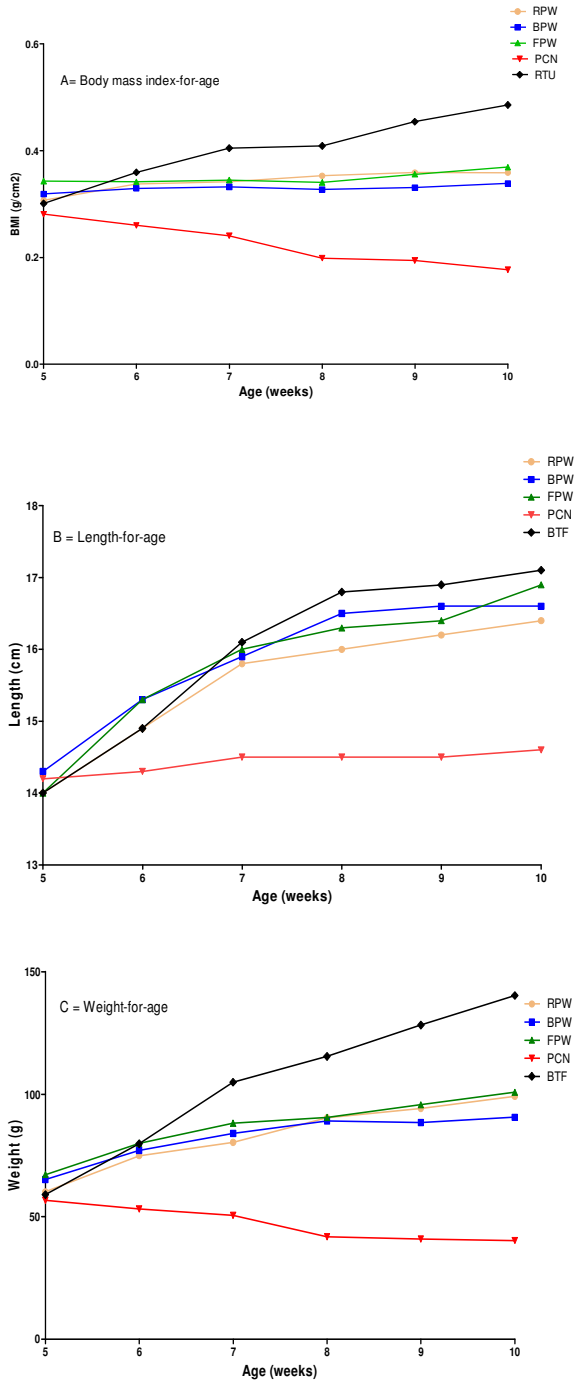

Fig. 1. Body mass index-for-age, Length-for-age and Weight-for-age of Wistar rats fed experimental complementary foods and BTF (a therapeutical food produced by UNICEF)

The body mass index (BMI) (a measure of underweight/obese) of the rats fed on formulated complementary foods (0.34 - 0.37 g/cm^2) were significantly ($p<0.05$) higher than those rats fed on PCN (a popcorn flour sample) (0.18 g/cm^2), but lower than those rats fed on BTF (a UNICEF formulated diet) (0.49 g/cm^2). Comparatively, the BMI of rats obtained in this study were lower than those values reported by Engelbregt et al. (2001) who investigated the body composition of pubertal male rats and reported the BMI of 0.53 g/cm^2, and Novelli et al. (2007) who reported BMI of normal adult rats to range from 0.45 to 0.68 g/cm^2. Weight-for-age (WFA) (underweight, a measure of chronic and acute malnutrition) showed that those rats fed with experimental diets had better growth patterns when compared to those rats fed on PCN sample, but they were comparable to those rats fed on BTF diet. The classification of rats using length-for-age nutritional index showed that those rats fed on experimental complementary foods had better growth patterns when compared to those rats fed on PCN sample, but lower than those rats fed on BTF. Nutritionally, it could be deduced from this study that these formulated experimental foods were suitable as complementary foods in providing essential nutrients for the physiological needs of children.

In vivo protein digestibility of complementary foods produced from popcorn, soybean cake and wonderful kola flour blends

The protein qualities of formulated complementary foods are presented in Table 6. The weight gained by the rats fed on experimental complementary foods with range values of 25.4±0.04 – 33.6±0.15g were significantly ($p<0.05$) higher than those rats fed on PCN (-6.4±0.15g), but lower than those fed on control (BTF) sample (81.3±0.10g). This observation may be due to high fat content of BTF, which was purposely added during formulation in order to increase the energy value of the product.

Table 6: Protein qualities of experimental food samples

Parameters	RPW	BPW	FPW	PCN	BTF
Weight gained (g)	29.0^{c}±0.2	25.4^{d}±0.04	33.6^{b}±0.15	-6.4^{e}±0.15	81.3^{a}±0.1
FE	0.12^{c}±0.01	0.10^{d}±0.1	0.14^{b}±0.02	-0.03^{e}±0.01	0.33^{a}±0.02
NR	6.39^{b}±0.01	5.04^{c}±0.02	7.80^{a}±0.01	0.27^{e}±0.01	3.77^{d}±0.01
BV (%)	69.45^{d}±0.045	70.74^{c}±0.025	78.41^{b}±0.015	13.08^{e}±0.015	84.99^{a}±0.01
NPU (%)	60.61^{c}±0.01	53.75^{d}±0.01	70.58^{a}±0.02	10.63^{e}±0.03	63.57^{b}±0.01
TD (%)	87.27^{a}±0.15	75.98^{c}±0.02	75.99^{c}±0.01	81.26^{b}±0.02	74.79^{d}±0.05
PER	0.44^{c}±0.02	0.43^{c}±0.01	0.49^{b}±0.02	-0.39^{d}±0.01	2.19^{a}±0.05
PR	1.036^{c}±0.01	0.91^{d}±0.01	1.20^{b}±0.02	-0.23^{e}±0.02	2.90^{a}±0.03

Mean± SEM across the rows with different superscripts differ significantly at P<0.05.

Table 7: Weight of liver, heart, kidney and carcass of experimental food samples

Parameters	Liver	Heart	Kidney	Carcass
RPW	5.70^{b}±0.10	0.44^{b}±0.03	1.15^{b}±0.15	74.25^{c}±0.25
BPW	4.85^{c}±0.25	0.62^{a}±0.01	1.20^{a}±0.10	70.35^{d}±0.25
FPW	3.80^{d}±0.10	0.32^{c}±0.01	0.90^{c}±0.10	77.20^{b}±0.20
PCN	2.65^{e}±0.25	0.33^{c}±0.02	0.64^{d}±0.04	38.30^{e}±0.50
BTF	6.20^{a}±0.15	0.40^{b}±0.02	0.90^{c}±0.25	107.3^{a}±2.52

Mean± SEM across the rows with different superscripts differ significantly at P<0.05.

The biological values (BV) of experimental food samples ranged from 69.45% in RPW to 78.41% in FPW, and were significantly lower than BTF (a control sample) (84.99%). The disparity between the BV of experimental diets and BTF may be due to their different in food composition. For instance, BTF was produced from wheat, oat, sugar, vegetable oil, vegetable protein and skimmed milk powder which are not applicable to the present study formulations. It is well known that animal-based foods, such as milk, are usually high in biological value and essential amino acids than in plant-based food products. The protein efficiency ratio (PER) of complementary foods varied from 0.43 in BPW to 0.49 in FPW, while protein rating (PR) of the food samples had FPW (1.20) and BPW (0.91) as the highest and least respectively, and these values were significantly ($p<0.05$) lower than BTF, but higher than in PCN. The BV of complementary foods in this present study was similar to the report of Abiose *et al.* (2015), who reported on the biological values of complementary foods from fermented and malted quality protein maize fortified with soybean flour. The BV and PER of the formulated food samples met FAO/WHO (1989) recommended values of 70% for BV, but lower to 2.7 for PER. This implies that the protein in the experimental food samples may be adequate to support growth and development in infant, and also to maintain other physiological needs in children and adults.

The weight of liver, heart, kidney and carcass of rats fed on experimental complementary foods showed that RPW had the highest values for liver, heart and kidney, but low in carcass, whereas, FPW had the lowest weight for liver, heart and kidney but higher in carcass (Table 7). In comparing with control samples, it was observed that the experimental functional foods had better weights of liver, heart, kidney and carcass than for PCN, but lower than that of BTF sample. This implies that the diets were suitable for the development of these organs without any side effects in rats.

Haematological properties of rats fed on formulated complementary foods

Haematological properties of rats fed on formulated complementary foods is shown in Table 8. The pack cell volume (PCV) of the experimental rats fed on FPW sample (47.2%) had the highest concentration, while RPW (38.0%) had the lowest concentration.

Table 8. Haematological indices of Wistar rats fed on formulated complementary foods

Parameters	RPW	BPW	FPW	PCN	BTF	Normal range
ERS (mm^3)	0.2^c	0.5^b	0.5^b	2.0^a	0.5^b	-
PCV (%)	38.0^b	47.0^a	47.2^a	35^c	48^a	30-50
RBC ($x10^6mm^3$)	9.78^b	14.48^a	14.59^a	7.22^c	14.72^a	4 – 8
WBC ($x10^3mm^3$)	16.4^a	14.85^c	15.80^b	3.42^d	3.35^d	5-12
Hb (g/dL)	12.7^c	15.7^b	15.9^b	11.7^d	16.0^a	8-17.5
Lymphocytes (%)	62^b	63^a	63^a	56^c	63^a	25-50
Neutrophils (%)	29^a	28^a	26^b	27^b	27^b	36-55
Monocytes (%)	6.0^c	6.0^c	8.0^a	7.0^b	7.0^b	-
Eosinophils (%)	2.0^a	2.0^a	2.0^a	2.0^a	2.0^a	0-5
Basophils (%)	1.0^a	1.0^a	1.0^a	1.0^a	1.0^a	-
MCV (µm)	10.23^b	6.91^c	6.85^c	13.85^a	6.79^c	82-98
MCH (pcg)	12.99^b	10.84^c	10.76^c	16.21^a	10.87^c	26-34
MCHC (g/dL)	33.42^a	33.4^a	33.4^a	33.43^b	33.33^c	31-37
Colour index	1.30^a	1.08^b	1.08^b	1.09^b	1.03^c	0.8-1.2

Mean± SEM across the rows with different superscripts differ significantly at P<0.05.

The red blood cell (RBC) varied between 9.78 x 10^6mm^3 in RPW and 14.59 x 10^6mm^3 in FPW, heamoglobin (Hb) concentrations ranged from 12.7 g/dL) in RPW to 15.9 g/dL in FPW, while white blood cells concentration ranged from 14.85 in BPW to 16.4g/dL in RPW. Comparatively, the PCV, RBC and Hb concentration of the rats fed on experimental complementary foods were lower than those values obtained for BTF sample, but higher than those rats fed on PCN. The hematological variables observed in this present study were similar in terms of PCV, RBC, MCV, MCHC and lymphocytes to the reports of Fayomi *et al*. (2014) and Okoruwa *et al*. (2014). It is well known that hematological indices are important variables to determine the health status of a subject. For instance, the level of RBC counts helps in the characterization of anaemia (Ikhimioya and Imasuen, 2007), while varying in lymphocyte values will indicate different levels of immune status in man (Aikuomobhogbe and Orheruata, 2006; Adua *et al.,* 2015). The haematological profile in this study further indicates the nutritional qualities of the formulated diets, for instance, the elevated level of PCV, RBC and Hb concentration in rats fed on experimental diets are indicated that the complementary foods were very rich in protein, minerals and vitamins which aided blood formation. Roberts *et al*. (2000) and Oluwole *et al.* (2001) reported that Hb, PCV and MCHC concentration depend on the quantity and quality of dietary protein intake; and that diets containing poor protein may result into poor haemoglobin formation.

Sensory Attributes of formulated complementary foods

The aroma, colour, taste, texture and overall acceptability parameters of the formulated food samples were significantly ($p<0.05$) rated lower by the panelists compared to control sample (Figure 2).

The disparity between the formulated diets and control food sample in terms of taste, aroma and overall acceptability could be attributed to the familiarity of the panelists with the control food sample over the new formulated products. The differences between fermented functional food sample (FPW) and other products in overall acceptability could be due to different processing techniques. For instance, it has been scientifically proven that fermentation positively improve organoleptic properties and nutritive values of food products (Ochanda *et al*., 2010).

To improve on the sensory attributes of the present study formulations, there is a need to add sweeteners and flavourant.

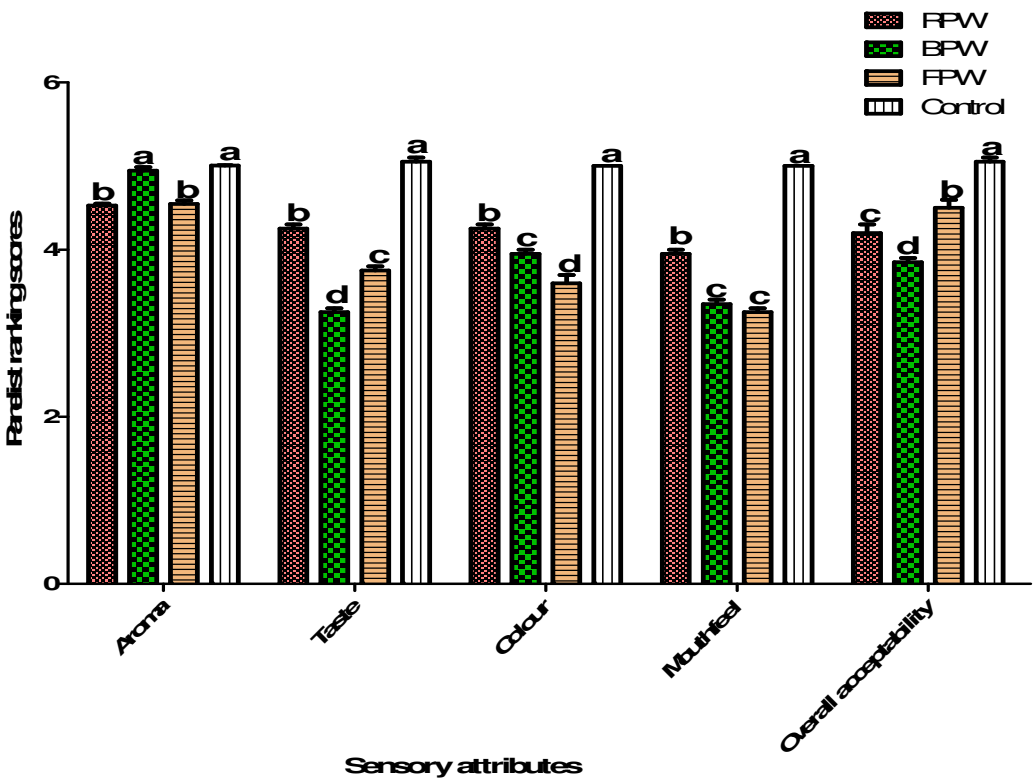

Fig. 2. Sensory attributes of formulated complementary foods and BTF (a therapeutical food by UNICEF).

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