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Mineral bioavailability and chemical composition of cereal-based complementary food

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The aim of this research was to produce a nutrient-rich cereal-legume based complementary food with high mineral bioavailability fortified with micronutrients from locally available sources. Maize-malted cowpea $(MC_m)_b$ and maize-malted bambara nut $(MB_m)_b$ complementary foods were fortified with calcium (Ca), iron (Fe), zinc (Zn) and provitamin A using processed cattle bone, roselle and red palm oil (mixed with *Brachystegia eurycoma (achi)* respectively in a modified post-fermentation process by wet-mixing. NUTREND, a commercial complementary food, served as control. The proximate, Fe and Zn contents were above the recommended daily allowance (RDA) except for Ca which was 0.054 mg/100 g [for $(MB_m)_b$ and $(MC_m)_b$] as against 2.5 – 5.0 mg/100 g and provitamin A which was 3.93 and 3.36 µgRE/100 g for $(MB_m)_b$ and $(MC_m)_b$ respectively as against 5.00 µgRE/100 g. The quantity of each food fortificant was based on the level of the required nutrient as analysed and the RDA for the nutrient for 6 to 12-month-old infants. The quantified anti-nutrients (tannin, phytate and oxalate) were below the safe levels. The phytate: mineral molar ratios for Ca, Fe and Zn for $(MB_m)_b$ and $(MC_m)_b$ were by far lower than the maximum suggested desirable levels which suggested high relative mineral bioavailability.

Key words: Phytate:mineral molar ratio, iron, zinc, malting, fermentation.

INTRODUCTION

Infancy is the period within the first year of life during which growth is at its maximum such as cognitive, anatomical and physical development, as a result, there are very high nutritional requirements (Atkinson, 2013). Infants are vulnerable to malnutrition due to the relatively low food consumed as a result of the small capacity of their stomach, high demand for nutrients due to their body's fast developmental rate (Giugliani, 2019) and limited food choices (Román & Sánchez-Siles, 2018). Romero-Velarde *et al.,* (2016) reported that inadequate weaning food practices expose the child to health risks such as Fe and Zn deficiency. Protein-energy malnutrition in children between 0 to 12 months old especially in developing countries of the world is primarily caused by lack of nutrient-rich weaning foods with bioavailable micronutrients (Phu *et al.,* 2010).

Deficiency in protein and micronutrients (especially Fe) can have a lifelong effect (Dobe *et al*., 2018) in infancy and early childhood. This is prevalent in developing countries (Phu *et al*., 2010). About 20 million children worldwide suffer a nutrient deficiency, especially in South Asia and Sub-Saharan Africa (Ciliberto *et al*., 2005). India has the largest number of underweight children (Dobe *et al*., 2018), some African countries such as Rwanda have reported 44% level of stunted growth among children as a result of inadequate nutrient intake from complementary foods (Uwiringiyimana et al., 2018) while in rural Ethiopia, Abebe et al. (2017) reported 48 % similar scenario. But the good news is that micronutrient deficiency such as Fe deficiency can be managed through dietary improvement, supplementation and fortification (Lopez *et al.,* 2016). Dahdouh et al. (2019) classified Fe and Zn deficiency as a public health challenge and reported that about two billion people worldwide suffer from micronutrient deficiencies related illness, out of which 30% are anaemic while 17.3% suffer inadequate Zn intake. Complementary foods are nutrient-containing foods given to young children along with breast-milk within the 6 – 23 month window (Sawadogo *et al*., 2010). Barrera *et al*. (2018) reported that the American Academy of Pediatrics recommends the introduction of complementary foods to infants at six

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months of age and should not occur before four months of age. The early introduction might lead to obesity while late introduction has been associated with irregular eating patterns at later ages, micronutrient deficiency and allergies. Breastfeeding helps infants in developing more desirable eating behaviour (Lange et al., 2013). Micronutrient deficiencies especially of Ca, Fe, Zn and Vit. A are common during infancy (Attaugwu et al., 2016; Adu-Afarwuah *et al*., 2008; Gibson & Hotz, 2000). Ca helps in proper bone formation (Closa-Monasterolo *et al*., 2018), vitamin A helps to boost resistance to infections and for proper vision (Silverio *et al*., 2003), while Fe has been associated with psycho-motor development and proper functioning of the oxygen-carrying capacity of the blood (Doom *et al*., 2018; Blanco-Rojo & Vaquero, 2018). Zn, on the other hand, helps in normal growth and development in children and is vital for proper sense of taste and smell (Ackland & Michalczyk, 2016).

Cereals are the first complementary foods usually introduced to infants especially in developing countries. Consumption of fortified (Bartleman, 2019) complementary diets is therefore highly encouraged because the nutrient content of weaning foods in developing countries (Faber, 2005) are inadequate. The high cost of complementary foods especially containing animal product makes it out of reach to low-income earners. Some problems of single grain complementary food are that it contains insufficient quantities of the critical micronutrients (Ca, Fe and Zn) and the absorption of these micronutrients is often inhibited by the high levels of antinutrient factors in most traditional diets (Israr *et al*., 2017). Very frequent consumption of cereal-based complementary foods can lead to high risk of mycotoxin contamination, especially where the raw materials are exposed to poor postharvest management (Ortiz *et al*., 2018). However, Nyamete *et al*. (2016) reported that fermentation by back-slopping reduced aflatoxins in foods by 68% in maize-based gruel. Cereals such as maize and legumes are locally available in different communities globally and are mostly used in complementary feeding in Africa. The beany flavour of legumes such as cowpea and Bambara nut limits their use in complementary foods, however, fermentation, roasting and germination (malting) can considerably reduce the beany flavour (Kaczmarska *et al*., 2018). One of the ways to deliver more vitamins and minerals to infants is through fortification of foods. Work in our laboratories has shown that Fe and Zn contents of post-fermentation (wet-mix) fortified $(MC_m)_b$ and $(MB_m)_b$ complementary foods were lower than RDA. Plant-based complementary foods fall below the RDA of micronutrients especially Fe and Zn for 6 - 12 months old infants. Attempts to improve Ca, Fe, Zn and provitamin A in $(MB_m)_b$ and $(MC_m)_b$ complementary foods by food-to-food fortification showed that the best results were obtained by wet-mix fortification but the values for Fe and Zn (0.15 and 0.02 g/kg) were below the calculated requirements (Uvere *et al*., 2010). Fortification helps in delivering nutrient-dense diet thereby meeting the essential nutrient needs such as micronutrients (Mbuya & Neufeld, 2018). It has been indicated that the best way to deliver essential nutrients in recommended quantities is by using traditional diets which is affordable. The aim of this research was to produce a nutrient-rich cereal-legume based complementary food with high mineral bioavailability fortified with micronutrients from locally available sources

MATERIALS AND METHODS

Materials

Clean non-shrivelled mature seeds of Bambara nut (*Voandzeia subterranea* (*L*.) *Thouars*), cowpea [*Vigna unguiculata* (*L*.) *Walp*], cattle bone, roselle calyces [*Hibiscus sabdariffa* (*L*.) *malvaceae*], red palm oil, yellow maize [*Zea mays (L.) var. rugosa*], achi (*Brachystegia eurycoma*) and a factory manufactured infant formula (D) (a commercially available cereal-legume based infant formula, the brand name is NUTREND, manufactured by Nestle Nigeria Plc, 22 - 24, Industrial Avenue, Ilupeju, Lagos, Nigeria) were purchased from Nsukka main market, Nigeria.

Methods

The processing methods were based on the methods of Uvere *et al.* (2010) with some modification consisting essentially of the elimination of the fermentations of individual food fortificants prior to the formulation of the fortificant mix.

Production of maize-bambara and maize-cowpea food blends

The maize, bambara nut and cowpea seeds were cleaned by winnowing and hand sorting.

Production of degermed maize flour

The maize grains (700 g) were tempered in excess water for 15 mins, de-germed using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK), dried at $32 \pm 0.26^{o}C$, winnowed and the grits milled into flour using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK). The flour was packed in polyethylene bags and stored in the refrigerator at $4^{o}C$.

Production of bambara nut and cowpea flours

One lot (500 g) of bambara nut seeds was steeped in excess tap water at $28 \pm 0.56\ ^{o}C$ for 8 h, wet-dehulled by

Table 1. Ratio of maize to bambara nut malt/cowpea malt and fortificants.

Constituent	Ratio
Flour blend	**11.02**
Maize flour	2.33
Bambara nut /cowpea malt	1
Fortificants	**1**
Roselle Calyces ash	1
Cattle bone ash	5.51
Emulsified red palm oil (red palm oil, distilled water and *B. eurycoma* (1:1:2)	1.81

Table 2. Quantity of fortificant/flour blend (g) and water (ml) during fermentation.

DAY	Flour blend :	Water	Fortificant :	Water
	(60%)	(40%)	(60%)	(40%)
DAY 1	0.92	0.61	1.24	0.82
DAY 2	9.17	6.11	12.36	8.24
DAY 3	61.60	54.40	109.97	73.31
Total	71.69	61.12	123.57	82.37

abrasion between the palms and dried at 50^{o}C in a hot air Gallenkamp oven (Model IH-150, Gallenkamp, England). The dried grains were milled into flour using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK), packed in polythene bags and stored in the refrigerator at 4^{o}C.

Five hundred grams (500 g) of cleaned cowpea seeds were tempered in excess tap water at 28 ± 2^{o}C for 10 mins, wet dehulled by abrasion between the palms, dried in a Gallenkamp oven at 50 ^{o}C before milling into flour using the Bentall attrition mill. The flour was packed in a plastic container and stored in a refrigerator at 4^{o}C.

Lots of bambara nut seeds (200 g each) were weighed into porous malting bags (25 cm x 45 cm) for malting at 28±0.56^{o}C. The seeds were steeped in tap water for 8 h, air rested for 4 h and re-steeped in clean tap water for 8 h. The out-of-steep seeds were spread in malting bags and allowed to germinate in a dark room for 72 h during which they were turned once every 24 h. The samples were moistened on alternate days by dipping the malting bags containing the germinating grains in water for 30 secs. The green malts were dried in a convection Gallenkamp oven (Model IH-150, Gallenkamp, England) at 50^{o}C for 12 hrs after which the seeds were cleaned of sprouts and hulls by abrasion between the palms and winnowed. The malts were milled into flour using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK) and stored in polyethene bags in a refrigerator at 4^{o}C.

Lots of sorted cowpea seeds (500 g) were soaked for 4 h followed by 2 h air rest and another 4 h water steep before sprouting in the dark for 72 h in the malting bags. The malts were dried in a Gallenkamp oven (Model IH-150 Gallenkamp, England) and cleaned of sprouts. The dried malts were milled into the flour with a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK) packed in a plastic container and stored in a refrigerator at 4^{o}C.

Production of fermented composite flour blends

Composite flours from maize and malts of bambara nut/ cowpea were formulated in a 70:30 ratio (Table 1) (Cameron and Hofvander, 1983). The maize-bambara nut malt or maize-cowpea malt blends were fermented for 72 h by backslopping. The ratio of flour blend to water during fermentation by backslopping was 60:40 (Table 2).

Processing of foods used as fortificants

Processing of cattle bones into meal

Cattle bones used as a source of Ca was cracked open using Bench Vice Machine (Model HI-Duty Vice Paramo, England), washed with hot water at 90^{o}C to remove the marrow and oil, then dried in a Gallenkamp oven (Model IH-150, Gallenkamp, England) at 50^{o}C for 12 hrs. The dried bones (100 g) were then ashed at 600^{o}C for 5 h.

Processing of roselle calyces into flour

Roselle calyces used as a source of Fe and Zn were

hand-sorted to remove dirt and extraneous materials. 100 g was dried at 50^{o}C milled into flour and ashed at 450^{o}C.

Processing of red palm oil

The red palm oil used as a source of provitamin A was processed by forming a 24 h stable emulsion with *Brachystegia eurycoma*. *B. eurycoma* seeds were roasted at 150^{o}C for 30 mins, soaked in excess water for 3 h, dehulled by abrasion and milled into powder using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK). The powder was used to form a 24 hr stable emulsion of red palm oil, water and *B. eurycoma* (1:1:2, v/v/w).

Formulation of fortificant mix

The quantity of each processed food fortificant in the mix (1:1.81:5.51 for roselle, emulsified red palm oil and cattle bone, respectively, (Table 1) was based on the level of the required nutrient as analysed and the RDA for the nutrient for 6 to 12 month old infants. Thereafter, the fortificant mix was fermented by backslopping for 72 h. The ratio of fortificant to water during the three days of fermentation by backslopping (Table 2) was 60:40.

Formulation of $(MB_m)_b$ and $(MC_m)_b$ complementary food blends

The fermented maize-bambara nut and maize-cowpea malt complementary foods were wet-mixed with the fermented fortificant mix in a ratio of 11.02 : 1 (Table 1) and dried in a convection Gallenkamp oven (Model IH-150, Gallenkamp, England) at 50 ^{o}C followed by milling into flour in a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK), packaged in polyethylene bags, sealed and stored at 4^{o}C.

Chemical analyses of the fortified complementary food blends

Proximate analysis

Moisture, ash, crude protein, crude fat, crude fiber contents were determined according to the procedures described in the AOAC (AOAC, 2010). The total carbohydrates were calculated by difference.

Micronutrient Analysis

Determination of Ca, Fe and Zn

Ca, Fe and Zn contents were determined according to the procedures described in the AOAC (AOAC, 2010).

Determination of provitamin A

Provitamin A was determined as described by (Biesalski *et al*., 1986). A stock solution was prepared by dissolving 25 mg Retinol in 85 ml Isopropanol (99%). Standard solutions of concentrations 0.0, 5.0, 10.0, 15.0 and 20.0 were prepared from this stock solution. The standard solution was used to prepare a calibration curve.

Twenty millilitres (20 mls) of Isopropyl alcohol was added to 5 g of the sample in a test tube and allowed to stand for 45 mins at room temperature (28+0.56 ^{o}C). The mixture was gently swirled and filtered using Whatman filter paper No. 1 (11 microns). The filtrate was measured at 325 nm for provitamin A using UV-Visible Spectrophotometer, Model 2550 (Shimadzu, Japan) located at NARICT (National Research Institute for Chemical Technology) Zaria, Kaduna state, Nigeria.

Determination of anti-nutrients

Tannin

The tannin content was estimated spectrophotometrically by the Folin-Denis method as described by Makkar *et al*. (1993). The method is based on the oxidation of the molecules containing a phenolic hydroxyl group. The tannin and tannin-like compounds reduce phosphomolybdic acid in alkaline solution to produce a highly coloured blue solution; the intensity of which is proportional to the amount of tannin and can be estimated against standard tannic acid solution at a wavelength of 725 nm.

Sample preparation and extraction of tannins

The sample was dried at 55±1^{o}C and ground to pass through a sieve of 1 mm diameter. Tannin extraction was done using 400 mg ground sample in a conical flask with 40 ml diethyl ether containing 1% acetic acid (v/v) and mixed to remove pigments. The supernatant was carefully decanted after 5 mins and then 20 ml of 70% aqueous acetone was added. The flask was sealed with cotton plug covered with aluminium foil and kept in an electrical shaker (Clarkson MX 001014 Helix 150 BLR, USA) for 2 h for extraction. Then it was filtered through Whatman filter paper No. 1 (11 microns) and the extract was kept in a refrigerator at 4^{o}C until analysis.

A standard calibration curve was prepared from the stock solution of tannic acid (0.5 mg/ml) using 0, 10, 20, 30, 40 and 50 µg respectively. Then 0.5 ml Folin reagent and 2.5 ml of 20% sodium carbonate was added and the contents mixed properly; and after 40 mins, the absorbance reading at 725 nm was read in a BIOCHROM 4049 UV spectrophotometer (BIOCHROM LTD. UK.) located at NARICT (National Research Institute for Chemical Technology) Zaria, Kaduna state, Nigeria.

50 µl of tannin extract for each sample was taken in a test tube and the volume made up to 1.0 ml with distilled water. Then, 0.5 ml Folin Ciocalteu reagent was added and mixed properly followed by 2.5 ml of 20% sodium carbonate solution. These were added, mixed and kept for 40 mins at room temperature (28±0.56^{o}C). The optical density was read at 725 nm in a BIOCHROM 4049 UV spectrophotometer and the concentration was estimated from the standard curve.
Calculation:

$$\% \text{ tannin} = \frac{A_n \times Df}{A_S \times w} \times 100$$

Where: A_n = absorbance of test sample; A_s = absorbance of standard tannic acid

C = concentration of standard tannic acid (mg/ml)

D_f = dilution factor = V_{ex}/V_a; W = weight of test sample (mg)

V_{ex} = total volume of extract; V_a = volume of extract analyzed

Phytic acid

The phytic acid was determined using the procedure described by Lolas & Markakis (1975). 2.0 g of the sample was weighed into 250 ml conical flask to which 100 ml of 2% hydrochloric acid was added and allowed to stand for 3 h before filtering. 50 mls of each filtrate was placed in 250 ml beaker and 107 ml of distilled water added in each case to give proper acidity.

10 mls of 0.3 % Ammonium thiocyanate solution was added into each solution as an indicator and titrated with standard iron chloride solution, which contained 1.95 mg iron per ml. The endpoint was slightly brownish-yellow persisting for 5 mins. The percentage of phytic acid was calculated using the formula:
Calculation:
% phytic acid = Y x 1.19 x 100
Where: Y = titre value x 1.95mg.

Oxalate

Oxalate was determined using the method described by Oke (1969). 2.0 g of the sample was digested with 10 ml of 6 M HCl for one hr and made up to 250 ml in a volumetric flask after which it was filtered using Whatman filter paper No.1 (11 microns).

The pH of each filtrate was adjusted with conc. NH_4OH solution until the colour of the solution changed from salmon pink to faint yellow. Thereafter, the solution was heated on a water bath to 90^{o}C and allowed to stand for 8 h and then the suspension was now centrifuged at 2500 rpm, after which the supernatant was decanted and precipitate completely dissolved in 10 ml of hot 20 % (v/v) H_2SO_4.

The total filtrate resulting from the dissolution in H_2SO_4 was made up to 300 ml. An aliquot of 125 ml of the filtrate was heated until near boiling point and then titrated against 0.05 M of standardized $KMnO_4$ solution to a faint pink colour which persisted for about 30 secs after which the burette reading was taken. The oxalate content was evaluated from the titre value.
The overall redox reaction is:

$$MnO_4^- + C_2O_4^{2-} + 8H^+ \rightarrow Mn^{2+} + 4H_2O + 2CO_2$$

Calculation

The oxalate content was calculated using the formula:

$$\frac{T \times Vme \times Df}{M_E \times Mf} \times 100$$

where T is the titre of $KMnO_4$ (ml), Vme is the volume-mass equivalent (ie. 1 cm^3 of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), D*f* is the dilution factor (VT/A = 2.4 where VT is the volume of titrating (300 ml) and A is the aliquot used (125 ml), M_E is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and M*f* is the mass of sample used.

The molar ratio of phytate to iron, zinc and calcium

The phytate to iron, zinc and calcium molar ratios were calculated by the following equation (Gibbs, 2010)

$$\text{Phytate: mineral molar ratio} = \frac{(phytate\ (mg)/phytate\ molecular\ weight)}{(mineral\ (mg)/mineral\ atomic\ weight)}$$

where phytate molecular weight = 660
Calcium atomic weight = 40.08
Iron atomic weight = 56
Zinc atomic weight = 65.4

Statistical Analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) in a completely randomized design (CRD); mean separation was by Duncan's New Multiple Range Test (Steel and Torrie, 1980) technique. Significance was accepted at $p < 0.05$.

RESULTS

The proximate composition of the $(MB_m)_b$ and $(MC_m)_b$ complementary food blends (Table 3) show that the moisture content of the samples was low [(3.35 and 2.95 for $(MB_m)_b$ and $(MC_m)_b$ respectively)].

However, the protein, fat and carbohydrate contents of the $(MB_m)_b$ and $(MC_m)_b$ complementary foods compared favourably with the RDA which suggests nutrient-dense complementary food.
The Fe, Zn and provitamin A content of the $(MB_m)_b$ and $(MC_m)_b$ complementary foods compares favourably with the RDA and other standards (Table 4) except for Ca. The samples are therefore high in these micronutrients.

Table 3. Proximate composition of complementary food blends and RDA.

Proximate content (mg/100g)	$(MB_m)_b$	$(MC_m)_b$	N	RDA			
				6-11mo (40g)[2]	6-23 mo (50g)[2]	12-23mo (60g)[2]	6-23mo (100g)[2]
Moisture	3.35	2.95	2.50	-	-	-	-
Crude protein	20.80	22.50	15.00	3- 4.5	3- 5.5	4- 6.5	6-11
Fat	14.05	11.65	9.00	4.20	6.30	8.20	12.70
Crude fibre	1.30	1.40	7.00	-	-	-	-
Carbohydrate	55.73	56.72	64.20	-	-	-	-
Ash	4.77	4.78	2.30	-	-	-	-

Results are the means of three replications. Three individual batches of each formulation were prepared after which an average was taken. Each batch represented one replication. (MBm)b = maize-bambara groundnut malt complementary food, fermented and fortified, (MCm)b = maize-cowpea malt complementary food, fermented and fortified, N= proprietary formula. Based on 24% of energy as fat for infants ages 6-11 months, 28% of energy as fat for children ages 12-23 months, and 26% of energy as fat for infants ages 6-23 months (Lutter and Dewey 2003).

Table 4. Calcium, iron, zinc (mg/100 g) and provitamin A (µgRE/100 g) content of complementary food blends and raw materials used in production.

Samples	Ca	Fe	Zn	Provitamin A
C_b	$0.0062^{d} \pm 0.01$	$4.82^{c} \pm 0.10$	$0.28^{i} \pm 0.01$	$1.38^{g} \pm 0.06$
B_u	$0.065^{c} \pm 0.01$	$1.29^{j} \pm 0.10$	$0.42^{h} \pm 0.00$	$1.64^{f} \pm 0.01$
B_m	$0.062^{d} \pm 0.01$	$1.83^{h} \pm 0.04$	$3.84^{a} \pm 0.03$	$1.68^{f} \pm 0.01$
C_u	$0.065^{c} \pm 0.01$	$1.95^{g} \pm 0.03$	$0.83^{d} \pm 0.01$	$0.87^{h} \pm 0.01$
C_m	$0.055^{fg} \pm 0.01$	$2.32^{e} \pm 0.13$	$0.69^{f} \pm 0.00$	$3.42^{d} \pm 0.01$
R	$0.064^{cd} \pm 0.01$	$2.88^{d} \pm 0.05$	$0.75^{e} \pm 0.00$	$1.98^{e} \pm 0.01$
M	$0.059^{ef} \pm 0.01$	$1.47^{i} \pm 0.03$	$0.50^{g} \pm 0.00$	$3.85^{c} \pm 1.11$
B_{eu}	$0.054^{g} \pm 0.01$	$0.17^{l} \pm 0.02$	$0.05^{j} \pm 0.00$	$1.72^{f} \pm 0.05$
P	$0.411^{a} \pm 0.01$	$0.77^{k} \pm 0.02$	$0.43^{h} \pm 0.02$	$19.58^{a} \pm 0.15$
P_e	$0.069^{b} \pm 0.01$	$2.21^{f} \pm 0.01$	$0.49^{g} \pm 0.01$	$6.61^{b} \pm 0.05$
$(MB_m)_b$	$0.054^{g} \pm 0.01$	$5.30^{b} \pm 0.06$	$1.58^{b} \pm 0.00$	$3.93^{c} \pm 0.03$
$(MC_m)_b$	$0.054^{g} \pm 0.01$	$7.08^{a} \pm 0.01$	$1.37^{c} \pm 0.01$	$3.36^{d} \pm 0.01$
D	3.900	0.100	0.060	4.500
RDA[+]	2.5 – 5.0	0.275	0.1 – 0.125	5.0
WHO[+]	5.25	0.11	0.028	3.5
IOM[+]	2.7	0.11	0.03	5.0
FAO/WHO[+]	4.0	0.093	0.041	4.0

Results are the means of three replications. Three individual batches of each formulation were prepared after which an average was taken. Each batch represented one replication. Values carrying different superscripts in the same column are significantly different ($p<0.05$). Cb = cattle bone meal, Bu = unmalted bambara groundnut, Bm = malted bambara groundnut, Cm = malted cowpea, Cu = unmalted cowpea, R = roselle, M = maize, Beu = *Brachystegia eurycoma*, P = palm oil, Pe = emulsified palm oil, (MBm)b = maize-bambara groundnut malt complementary food, fermented and fortified, (MCm)b = maize-cowpea malt complementary food, fermented and fortified, D = proprietary formula, RDA = Recommended Dietary Allowance, +(Lutter and Dewey, 2003), WHO : World Health Organization, IOM : Institute of Medicine, FAO : Food and Agriculture Organisation

The antinutrients content (tannin, oxalate, phytate) of the $(MB_m)_b$ and $(MC_m)_b$ complementary foods are well below the safe levels (Table 5) which implies that they are safe for infant consumption.

Table 5. Tannin, oxalate and phytate composition of $(MB_m)_b$ and $(MC_m)_b$ complementary food blends.

Samples	Anti-nutrients (mg/100g)		
	Tannin	Oxalate	Phytate
$(MB_m)_b$	$0.315^{e} \pm 0.005$	$0.002^{c} \pm 0.000$	$0.076^{a} \pm 0.001$
$(MC_m)_b$	$0.380^{d} \pm 0.001$	$0.002^{c} \pm 0.000$	$0.0068^{b} \pm 0.001$
B_u	$0.478^{a} \pm 0.001$	$0.003^{b} \pm 0.001$	$0.007^{c} \pm 0.001$
B_m	$0.456^{c} \pm 0.000$	$0.001^{d} \pm 0.000$	$0.007^{c} \pm 0.000$
C_u	$0.463^{b} \pm 0.001$	$0.004^{a} \pm 0.000$	$0.005^{e} \pm 0.001$
C_m	$0.308^{f} \pm 0.000$	$0.001^{d} \pm 0.001$	$0.006^{d} \pm 0.000$
SL^{+}	150 – 200	400 – 500	301

Results are the means of three replications. Three individual batches of each formulation were prepared after which an average was taken. Each batch represented one replication. Values carrying different superscripts in the same column are significantly different ($p<0.05$). (MBm)b = maize-bambara groundnut malt complementary food, fermented and fortified (MCm)b = maize-cowpea malt complementary food, fermented and fortified, Bu = unmalted bambara groundnut, Cu = unmalted cowpea, MB = malted bambara groundnut, MC = malted cowpea, SL = safe level + Phytate: Heaney *et al.* (1991), Oxalate: Oke O.L. (1969), Tannin: Schiavone *et al.* (2007).

Table 6. Phytate:mineral molar ratio of $(MB_m)_b$ and $(MC_m)_b$ complementary food blends.

Samples	Phytate (mg/100g)	Minerals (mg/100g)			Phytate:Mineral molar ratio		
		Ca	Fe	Zn	Phytate: Ca	Phytate: Fe	Phytate: Zn
$(MB_m)_b$	0.00076	98.69	5.30	1.58	8.6×10^{4}:1	1.2×10^{5}:1	4.8×10^{5}:1
$(MC_m)_b$	0.00068	100.70	7.08	1.37	7.6×10^{5}:1	8.1×10^{6}:1	4.9×10^{5} :1
SDL^{+}					< 1	< 18	< 0.17

Results are the means of three replications. Three individual batches of each formulation were prepared after which an average was taken. Each batch represented one replication. (MBm)b = maize-bambara groundnut malt complementary food, fermented and fortified, (MCm)b = maize-cowpea malt complementary food, fermented and fortified +Gibson *et al* (2010), SDL: suggested desirable levels; molar mass of phytate = 660, calcium = 40.08, iron = 56, zinc = 65.4.

The low phytate:mineral molar ratios (Table 6) which is below the suggested desirable levels suggest that the relative mineral bioavailability of $(MB_m)_b$ and $(MC_m)_b$ complementary foods would be high when consumed by infants.

DISCUSSIONS

Proximate composition of $(MB_m)_b$ and $(MC_m)_b$ complementary food blends

The low moisture content of the $(MB_m)_b$ and $(MC_m)_b$ complementary food blends (Table 3) of less than 3.5% could be due to hydrolysis of macromolecules such as starch and protein during malting and fermentation resulting in loss of water holding capacity. However, the moisture content of the proprietary formula was lower (2.50 %) than that for $(MB_m)_b$ and $(MC_m)_b$.

The crude protein content of the $(MB_m)_b$, was 20.80 mg and for $(MC_m)_b$, 22.50 mg. These values were higher than the 15.00 % value for the proprietary formula. The higher crude protein content could be attributed to microbial biomass and additional contributions from the crude protein content (9.98 %) of *B. Eurycoma* (Uzomah and Odusanya, 2011), the 17.70% of Bambara nut (Eltayeb, 2011) and 24.80% of cowpea (Davis et al*.,* 1991).

The sample, $(MB_m)_b$ contained a higher amount of fat than $(MC_m)_b$, (14.05 vs 11.65 mg) probably because bambara nut contains more fat than cowpea. The fat content of the blends was higher than that of the proprietary formula (9.00 mg) which could be due to contributions from red palm oil and could improve provitamin A absorption from the food. Bambara nut contains about 6.58 % of fat (Eltayeb et al., 2011) while

cowpea contains between 0.60 and 1.90 % (Davis *et al*., 1991, Henshaw, 2008).

The ash content of the $(MB_m)_b$ and $(MC_m)_b$ were 4.77 mg/100g and 4.78 mg/100g, respectively, and were higher ($p<0.05$) than that for the proprietary formula (2.30 mg/100g). This increase might be attributed to the effect of fortification and loss of organic matter during fermentation (Obizoba and Atti, 1991).

The low fibre contents of 1.3 mg for $(MB_m)_b$ and 1.4 mg for $(MC_m)_b$ may be due to the use of dehulled raw materials in the formulation. The fiber content of the proprietary formula, (7.0), was considerably higher than 1.3 mg for $(MB_m)_b$ and 1.4 mg $(MC_m)_b$.

The carbohydrate content of 55.73 mg/100g and 56.72 mg/100g for $(MB_m)_b$ and $(MC_m)_b$, respectively were lower compared to the 64.2 mg/100g for the proprietary formula.

Micronutrient composition of $(MB_m)_b$ and $(MC_m)_b$ complementary food blends

The calcium content of the $(MB_m)_b$ and $(MC_m)_b$ complementary foods were considerably low [$(MB_m)_b$ = 0.0535 mg/100g; $(MC_m)_b$ = 0.0544 mg/100g] compared with the RDA of 2.50 – 5.00 mg/100g. (Table 4). These low values for calcium may be due to calcium utilization by fermenting microorganisms (Adewusi et al*.,* 1999). This derives from the fact that the calcium content of $(MB_m)_b$ (0.0621 mg/100g), $(MC_m)_b$ (0.0554 mg/100g) and maize (0.059 mg/100g) were higher than the calcium content of $(MB_m)_b$ and $(MC_m)_b$.

The iron content of $(MC_m)_b$ complementary food was significantly ($p<0.05$) higher than that of $(MB_m)_b$ complementary food (7.08 mg/100g vs 5.30 mg/100g) (Table 4). Both $(MC_m)_b$ and $(MB_m)_b$ were significantly ($p<0.05$) higher than that in the proprietary formula (0.100 mg/100g), and the recommended dietary allowance for 6-23 month infants (0.125 mg/100g). The high iron content derives from the effect of concentration during malting of the legumes, contributions from processed roselle, maize and *B. eurycoma* used to emulsify palm oil. The higher iron content of $(MC_m)_b$ may, therefore, be due to the higher iron content of cowpea.

In the case of zinc, however, $(MB_m)_b$ had a significantly higher content of 1.58 mg/100g than $(MC_m)_b$ with 1.37 mg/100g (Table 4). Both values were higher than RDA (0.125 mg/100g) and the value for the proprietary formula (0.060 mg/100g). The increased Zn contents derive from the same reasons adduced for iron content. But $(MB_m)_b$ had a higher Zn content than $(MC_m)_b$ because of the higher zinc content of bambara nut malt (3.84 mg/100g). The Fe and Zn contents of $(MB_m)_b$ [5.30 mg/100g; 1.58 mg/100g] and $(MC_m)_b$ [7.08mg/100g; 1.37mg/100g] were significantly ($p<0.05$) higher than the 0.134 mg/100g and 0.015 mg/100g reported by Uvere et al. (2010).

The provitamin A content of 3.93 µgRE/100g in $(MB_m)_b$ was significantly higher than the 3.36 µgRE/100g of $(MC_m)_b$ in spite of the significantly higher provitamin A content in cowpea malt (Table 4). This suggests that $(MC_m)_b$ may have components that sequester provitamin A. The values were however less than RDA (5.00 µgRE/100g) and the proprietary formula (4.50 µgRE/100g). Palm oil contained the highest amount of provitamin A (19.58 µgRE/100g) but the emulsified palm oil contained a lower amount of 6.61 µgRE/100g. This suggests that emulsification with *B. eurycoma* reduces the quantity of provitamin A. Malting increased the provitamin A content of bambara nut from 1.64 to 1.68 µgRE/100g and cowpea from 0.87 to 3.42 µgRE/100g.

Anti-nutrients composition of $(MB_m)_b$ and $(MC_m)_b$ complementary food blends

The oxalate, phytate and tannin contents of $(MB_m)_b$, were 0.315, 0.002, and 0.076 mg/100g and for $(MC_m)_b$ 0.0068, 0.38, 0.002 and mg/100g, respectively (Table 5). These low values possibly derived from the low content of these anti-nutrients in the unprocessed raw materials, dehulling of bambara nut and cowpea, degerming of maize, malting of bambara nut/cowpea and fermentation (Obizoba and Atti, 1991).

The tannin content of $(MC_m)_b$, 0.380 mg/100g was higher than that of $(MB_m)_b$, 0.315 mg/100g in spite of the higher tannin content of B_m (0.456) compared to C_m (0.308) suggesting that fermentation may have more reductive effect on cowpea malt tannins than on bambara nut malt or that it could be due to effects relating to the fortificant mix. The low tannin content of the $(MB_m)_b$ and $(MC_m)_b$ complementary food could be attributed to the activity of malt enzymes. Similar results were reported by Obizoba and Egbuna (1992).

The decrease in oxalate content of the $(MB_m)_b$ and the $(MC_m)_b$ complementary foods could be as a result of steeping and fermentation as reported by Quinteros et al. (2003). Malting reduced oxalate contents of bambara nut and cowpea by 0.002 and 0.003 units, respectively, suggesting that the oxalate contents of $(MB_m)_b$, 0.002 mg/100g and $(MC_m)_b$, 0.002 mg/100g may be due to contributions from the fortificant mix or due to concentration as a result of fermentation of $(MB_m)_b$ and $(MC_m)_b$.

Malting had no effect on the phytate in bambara nut but increased that of cowpea. The phytate in $(MB_m)_b$, 0.076 mg/100g was significantly ($p<0.05$) higher than $(MC_m)_b$, 0.0068 mg/100g and were significantly less than the safe levels of 300. The phytate content of $(MC_m)_b$ was significantly ($p<0.05$) lower than that of $(MB_m)_b$ while the tannin contents were in a reverse relationship.

Phytate:mineral molar ratios of $(MB_m)_b$ and $(MC_m)_b$ complementary food blends

The $(MB_m)_b$ and $(MC_m)_b$ complementary foods had

phytate:mineral molar ratios (Table 6) which were by far lower than the maximum suggested desirable levels and suggests that the relative mineral bioavailability of $(MB_m)_b$ and $(MC_m)_b$ would be high (Gibson et al*.,* 2010; Gibson and Hotz, 2000).

$(MB_m)_b$ had phytate:mineral molar ratios of 8.6×10^4, 1.2×10^5, 4.8×10^5 for Ca, Fe and Zn. $(MC_m)_b$ had 7.6×10^5, 8.1×10^6, 4.9×10^5 for the respective minerals in the samples. $(MB_m)_b$ had higher ratios for Fe and Ca than $(MC_m)_b$, while $(MC_m)_b$ had a higher ratio for Zn than $(MB_m)_b$.

Conclusions

Fortified maize-bambara nut malt and maize-cowpea malt complementary foods for infants could be produced using a combination of household technologies such as malting, fermentation, fortification and drying. The use of processed cattle bone, roselle calyces and palm oil as fortificants for calcium, iron, zinc and provitamin A, respectively, improved the Fe and Zn contents but did not improve the Ca and provitamin A contents. However, the processing methods were therefore effective. The modified post-fermentation (wet-mix) fortification method significantly reduced anti-nutrient contents below the safe levels of the $(MB_m)_b$ and $(MC_m)_b$ complementary foods. Animal studies is recommended to investigate the toxicity level, if any, and confirm the bioavailability of the micronutrients. Possibility of commercial applications and storage stability of the complementary food blends could be exploited.

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