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Production of maize–bambara groundnut complementary foods fortified pre-fermentation with processed foods rich in calcium, iron, zinc and provitamin A

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Abstract

BACKGROUND: Maize–bambara groundnut complementary foods are deficient in calcium, iron, zinc and vitamin A. Food-to-food fortification could be cheaper, safer and more easily adopted by local communities compared to the use of chemically pure compounds and vitamins to enrich such foods.

RESULTS: Maize–bambara groundnut complementary foods fortified for iron, zinc, calcium and vitamin A by blending with a multi-mix (1.41 : 1 : 2.25, w/w) of processed roselle calyces, cattle bones, and red palm oil in a 1 : 2.1 (w/w) ratio showed significant increases in calcium, iron, zinc and vitamin A contents of 3.26–4.225, 0.083–0.134 and 0.015–0.017 g kg^{-1} and 4855.3–7493.7 μgRE kg^{-1}, respectively.

CONCLUSION: The maize–bambara groundnut foods had calcium, iron, zinc and vitamin A contents that satisfy the proposed nutrient requirements for infants. Only the maize–bambara groundnut and maize–bambara groundnut malt fermented by backslopping [$(\mathrm{MB})_b$ and $(\mathrm{MB}_m)_b$] containing red palm oil emulsified with *Brachystegia eurycoma* had calcium contents significantly ($P < 0.05$) higher than Nutrend, a complementary food produced by Nestle (Nigeria) PLC. These products are from raw materials produced in commercial quantities by rural farmers using household level technologies which the rural and urban poor can more easily access in order to reduce micronutrient malnutrition.

Keywords: maize–bambara; food-to-food; fortification; pre-fermentation

INTRODUCTION

Complementary foods are given to infants in addition to breast milk to ensure adequate growth, prevent malnutrition, stunting and anaemia[1] when breast milk nutrients become inadequate for their energy and growth needs. Complementary feeding starts from 4 to 6 months of a child's life.[2] Poor complementary feeding is the immediate direct cause of malnutrition leading to growth faltering and high rate of infections during infancy and early childhood.[3]

Ideal complementary foods are expected to have an easy-to-swallow, semi-liquid consistency of 1000–3000 mPa s,[4] have a good balance of amino acids, be rich in bio-available micronutrients and affordable. Cereal–legume mixes have been used in producing complementary foods in order to meet these requirements.[5,6] In spite of these and other attempts, problems associated with micronutrient inadequacies still persist and relate to deficiencies in calcium, iron, zinc and vitamin A. Vitamin A deficiency in children has been reported by the World Bank[7] to be endemic in Nigeria probably because its composition in complementary foods falls below the Recommended Dietary Allowance (RDA) for infants.

Food fortification with vitamin and mineral premixes and addition of milk solids has been used in many countries to address the problems of micronutrient deficiency.[8] The use of vitamin and mineral premixes results in products that are expensive to middle- and low-income earners, and sometimes fails to yield the desired results.

Work in our laboratory has shown that macronutrient-dense complementary foods could be produced from maize and bambara groundnut (70 : 30) with good physical and functional characteristics,[9] but their calcium, iron, zinc and vitamin A contents were below the recommended levels. Against this background, this study was designed to produce nutrient-dense complementary foods from maize and bambara groundnut and its malt and to increase the densities of calcium, iron, zinc and vitamin A by adding processed cattle bone, roselle calyces and palm oil before fermentation in order to meet the Recommended Dietary Allowance (RDA) for infants.

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EXPERIMENTAL

Materials

The bambara groundnut seeds (*Voandzeia subterranea* Thouars), yellow maize (*Zea mays)*, roselle calyces (*Hibiscus sabdariffa)*, cattle bones, *Brachystegia eurycoma*, red palm oil, potash (*akanwu*) and Nutrend were purchased from Nsukka market, Nigeria. *B. eurycoma* is a local soup thickener while potash is used as a palm oil emulsifier in the preparation of a local cassava snack (*abacha*). Red palm oil is traditionally processed without the use of solvent extraction or bleaching. Nutrend is produced by Nestle (Nigeria) PLC and was used as control.

Methods

Processing of raw materials

The maize and bambara groundnut seeds were cleaned by winnowing and hand sorting. The maize grains (700 g) were tempered in excess water for 15 min, de-germed using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK), sun-dried at 32 ± 0.26 °C to a moisture content of 10.5%, winnowed and the grits milled into flour. The flour was packed in polyethylene bags and stored at 4 °C.

One lot (500 g) of bambara groundnut seeds was steeped in excess tap water at 28 ± 0.56 °C for 8 h, wet-dehulled by abrasion between the palms and dried at 50 °C in a Gallenkamp oven (Model IH-150; Gallenkamp, England) to a moisture content of 11%. The dried grains were milled into flour, packed in polyethylene bags and stored at 4 °C.

Lots (200 g each) of bambara groundnut seeds were weighed into porous bags (25 cm × 45 cm) in which they were malted at 28 ± 0.56 °C by a modification of the two-step wet-steep method of EtokAkpan and Palmer.[10] The steeping schedule was based on the 14 h for maximum water absorption by undehulled bambara groundnut.[9] The seeds were steeped for 8 h, air rested for 4 h and re-steeped in clean tap water for 8 h. The seeds were spread in the 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 s. The root length was determined on a daily basis using a metre rule.

The green malts were dried in a convection Gallenkamp oven (Model IH-150) at 50 °C for 12 h to reduce its moisture content from 41% to 7.55%, after which the seeds were cleaned of sprouts and hulls by abrasion between the palms and winnowed. Malting loss was calculated as the loss in weight of bambara groundnut seeds due to steeping, germination and drying. The malts were milled into flour using a Bentall attrition mill (Model 200 L090) and stored in polyethylene bags at 4 °C.

Composite flours from maize and bambara groundnut or its malt were formulated in a 70 : 30 ratio.[11]

Processing of foods used as fortificants

Cattle bones. Cattle bones used as source of calcium were cracked open using Bench Vice Ma (Model HI-Duty Vice, Paramo, England), washed with water at 90 °C to remove the marrow and oil. It was dried in a convection Gallenkamp oven (Model IH-150) at 50 °C for 12 h and then transformed into bone meal as follows.

1. One hundred grams was autoclaved at 121 °C for 2 h, dried at 50 °C and milled into powder.
2. One hundred grams of cattle bone was milled and then fermented by backslopping[12] for 72 h before drying at 50 °C.
3. One hundred grams was ashed at 600 °C and milled.
4. One hundred grams was ashed at 600 °C, fermented by backslopping, dried at 50 °C and milled.
5. One hundred grams was fermented by backslopping for 72 h, dried at 50 °C and ashed at 600 °C before milling.
6. Another 100 g was autoclaved at 121 °C for 2 h and 1 g ashed at 600 °C.
7. A 100 g portion was autoclaved at 121 °C for 2 h and fermented by backslopping for 72 h, dried at 50 °C and 1 g ashed at 600 °C.
8. A 100 g portion was fermented by backslopping for 72 h, autoclaved at 121 °C for 2 h, dried at 50 °C and 1 g ashed at 600 °C.
9. A 100 g portion was autoclaved for 2 h, ashed at 600 °C and fermented by backslopping[12] for 72 h.
10. Raw bone (1 g) was dried at 50 °C, milled and used as control.

Roselle calyces. Roselle calyces *(Hibiscus sabdariffa*) used as a source of iron and zinc were hand-sorted to remove dirt and extraneous materials and processed as follows.

1. Four hundred grams was dried to a moisture content of 10% at 50 °C
   - a portion (100 g) of the dried calyces was ashed at 450 °C
   - 100 g portion was fermented by backslopping[12] for 72 h, dried at 50 °C and ashed at 450 °C.
2. A 100 g portion of fresh calyx was milled in a Bentall attrition mill (Model 200L090).
3. The fresh calyx (100 g) was fermented by backslopping for 72 h, dried at 50 °C and the resulting cake milled in a laboratory mortar.

Red palm oil. Red palm oil used as source of vitamin A was processed by forming 24 h stable emulsions with *B. eurycoma* (*achi*) and potash. *B. eurycoma* (*achi*) seeds were roasted at 150 °C for 30 min, soaked in excess water for 3 h, dehulled by abrasion and milled into powder. The powder was used in emulsification of red palm oil.

1. A 24 h stable emulsion of red palm oil, water and *B. eurycoma* (1 : 1 : 2, v/v/w) was formed, dried at 50 °C and the cake milled into flour.
2. A 24 h stable emulsion of red palm oil, water and *B. eurycoma* (1 : 1 : 2 v/v/w) was formed and fermented by backslopping for 72 h.
3. Half of the emulsified and fermented sample was dried at 50 °C and the cake milled in a laboratory mortar. Ground potash was also used to treat palm oil as described for *B. eurycoma*.

Incorporation of food fortificants

Red palm oil used as source of vitamin A was emulsified with *B. eurycoma* or potash. The *B. eurycoma* seeds were roasted for 30 min at 150 °C, soaked in excess water for 3 h, dehulled and washed. The cleaned seeds were milled, dispersed in water and used to form a 24 h stable emulsion with red palm oil (2 : 1 : 1; w/v/v) and dried at 50 °C for 24 h. Ground potash was also used to form a 24 h stable emulsion of water and red palm oil (2 : 1 : 1; w/v/v).

Cattle bones used as source of calcium were cracked open using Bench Vice Ma (Model HI-Duty Vice Paramo), washed with 90 °C hot water and dried in a Gallenkamp oven (Model IH-150) at 50 °C for 12 h. The dried bones were ashed at 600 °C and milled into powder in a Bentall attrition mill (Model 200 L090), packed in a plastic container and stored at 4 °C.

The roselle calyces which were the source of iron and zinc were hand-sorted, dried at 50 °C in a Gallenkamp oven (Model IH-150) to a moisture content of 8.5%, milled and ashed at 450 °C to a white powder which was packaged and stored at 4 °C.

The quantity of each processed food fortificant used was based on the level of the required nutrient as analysed in the processed food fortificant and the proposed RDA for the nutrient for 6- to 12-month-old infants.[13] The processed fortificants were mixed in the ratio of 1.41 : 1 : 2.25 (roselle calyces : cattle bone : emulsified red palm oil) on weight basis and mixed with the maize–bambara groundnut flour blends in a 2.1 : 1 (w/w) ratio. The mixture was fermented by backslopping[12] for 72 h and dried at 50 °C. The resulting cake was milled into flour using a hammer mill (H. Jurgens & Co., Bremen, Germany), packed in polyethylene bags, sealed and stored at 4 °C.

Analyses

Diastatic activity of the bambara groundnut malt was determined by the method of Hulse *et al.*[14] and expressed as degrees Lintner (°L). The maize–bambara groundnut mix (70 : 30) was analysed for oxalate content using the spectrophotometric method of Fassett[15] and the value expressed as mg oxalate 100 g^{-1}. Tannin content was determined by the method of Price *et al.*,[16] while phytate content was determined using the method of Latta and Eskin.[17] The proximate contents (moisture, crude protein, fat, fibre and ash) of the fortified maize–bambara groundnut blends were determined by the AOAC[18] methods; carbohydrate was estimated by difference. The processed cattle bone and the fortified maize–bambara groundnut blends were analysed for calcium according to the method described by Pearson;[19] vitamin A content in the processed palm oil and the fortified maize–bambara groundnut blends was determined by the method of Arroyave *et al.*[20] The processed roselle calyces and the fortified maize–bambara groundnut blends were analysed for iron content according to the method described by the AOCS,[21] while zinc was assayed for by the AOAC[18] method. Each analysis was repeated three times, unless otherwise stated. Data obtained were subjected to analysis of variance (ANOVA) and the means separated by Duncan's new multiple range test.[22] Significance was accepted at $P < 0.05$.

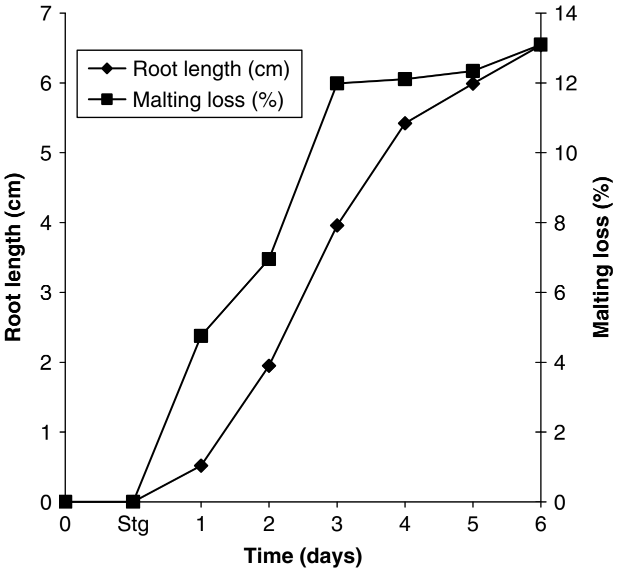

Figure 1. Root length and loss in weight of malting bambara groundnut seeds. Stg, out-of-steep grains.

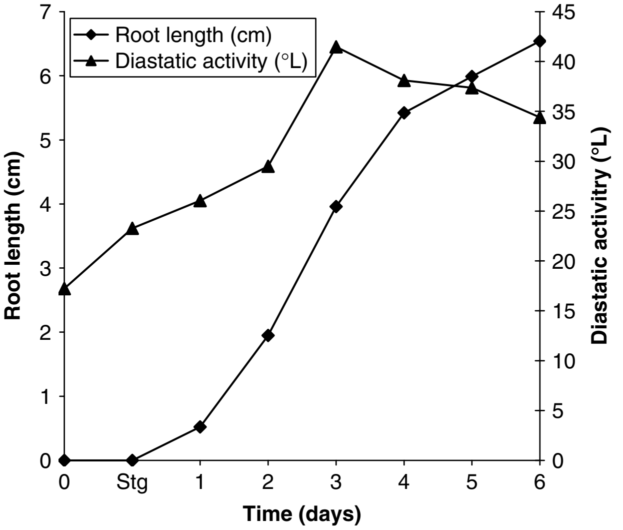

Figure 2. Root length and diastatic activity of malting bambara groundnut seeds. Stg, out-of-steep grains.

RESULTS AND DISCUSSION

Malting of bambara groundnut seeds

The root length and malting loss of bambara groundnut malts are shown in Fig. 1. The results show that the root length increased with germination time. The highest increase in root length between successive days (2.01 cm or 87.69%) was observed after 72 h (3 days) of malting while the first day had the lowest. The highest increase suggests improved modification of endosperm resulting from enhanced hydrolytic enzyme secretion and activity[23] and was significantly ($P < 0.05$) higher than other malting days. This could explain the increasing malting loss, which peaked at 11.86% after 72 h (Fig. 1) and could be attributed to respiration of the embryo, outgrowth of the root and shoot,[24] leaching of nutrients during steeping and degree of grain modification during germination.[25]

The diastatic activity (Fig. 2) increased as the duration of germination increased, with the day 3 malts having the highest value (41.47 °L). The high diastatic activity may be attributed to increased secretion, the extent of breakdown of granule cell wall and endosperm protein leading to subsequent hydrolysis of starch. Maduko,[9] similarly observed peak diastatic activity of bambara groundnut (43.56 °L) on malting day 3.

Effect of processing on selected micronutrient content of food fortificants

Calcium content of processed cattle bone

The calcium content of the processed cattle bone (Table 1) ranged from 3.36 ± 0.283 mg kg^{-1} in the raw bone to 7.64 ± 0.212 g kg^{-1} in the autoclaved, ashed and fermented (aAF) samples. Ashing followed by fermentation (AF) caused the release of 4.24 g/kg^{-1} calcium. Compared to ashing (A) alone, the relatively low increase of 0.80 g kg^{-1} shows that fermentation may not be an effective method of decomposing calcium hydroxyapatite. The higher

Table 1. Calcium content of processed cattle bone samples

Treatment	*Calcium content (g kg^{-1})	*Calcium content released (g kg^{-1})
C	$3.36^{a} \pm 0.283$	0
A	$3.40^{b} \pm 0.353$	0.04
F	$5.20^{c} \pm 0.141$	1.84
A	$6.80^{g} \pm 0.141$	3.44
FA	$5.72^{d} \pm 0.071$	2.36
aA	$6.08^{e} \pm 0.000$	2.72
AF	$7.60^{h} \pm 0.071$	4.24
FaA	$6.32^{f} \pm 0.000$	2.96
aFA	$6.32^{f} \pm 0.141$	2.96
aAF	$7.64^{i} \pm 0.212$	4.28

* Results are the means of duplicate determinations.
Values carrying different superscripts in the same column are significantly different ($P < 0.05$).
C = raw bone (control); a = autoclaved bone; A = ashed bone; F = bone fermented by backslopping; FA = bone fermented by backslopping and then ashed; aA = bone autoclaved and ashed; AF = bone ashed and fermented by backslopping; FaA = bone fermented by backslopping, autoclaved and ashed; aFA = bone autoclaved, fermented by backslopping and ashed; aAF = bone autoclaved, ashed and fermented by backslopping.

calcium released in the AF sample (4.24 g kg^{-1}) compared to the FA (2.36 g kg^{-1}) may be due to improved accessibility to the calcium hydroxyapatite as a result of the increased surface area provided by milling of the ashed sample before fermentation. The introduction of autoclaving (a) as a third treatment (aAF) gave the highest calcium release of 4.28 g kg^{-1}, which marginally differed from the 4.24 g kg^{-1} of the AF sample. The marginal difference of 0.04 g kg^{-1} between aAF and AF samples, though statistically significant ($P < 0.05$) may not be economical in terms of energy used; this suggests AF as the choice method of processing cattle bone into meal.

Iron and zinc contents of processed roselle calyces

The iron content (Table 2) ranged from 0.017 g kg^{-1} for the fresh leaves (moisture content = 6.875 g kg^{-1}) to 0.056 g kg^{-1} for the dried roselle calyces fermented by backslopping (RC$_{db}$; moisture content = 1 g kg^{-1}). The iron content of the dried and fermented roselle (RC$_{db}$ = 0.056 g kg^{-1}), suggests that some of the fermenting microorganisms associated with the fresh leaves which may require iron for their metabolism may have been eliminated during the initial drying process. This may be why drying and fermentation by backslopping gave a value less than would be expected from the combined effects of the individual treatments. The significant ($P < 0.05$) increase in the iron content of RC$_{db}$ over RC$_{d}$ (0.004 g/kg) may be attributed to phytate reduction during fermentation.[25] Babalola *et al.*[26] and Nnam and Onyeke[27] reported higher iron values of 0.378 g kg^{-1} and 8.33 g kg^{-1} during the fermentation of roselle calyces dried at 60 °C and 55 °C, respectively, which may be attributed to variations in soil fertility, the higher drying temperatures, fermentation and analytical methods.

The zinc content (Table 2) of the processed roselle calyces ranged from 0.022 g kg^{-1} for the fresh leaves with a moisture content of 68.75% to 0.043 g kg^{-1} for roselle calyces dried and fermented by backslopping (RC$_{db}$). The zinc content of the dried and fermented sample (RC$_{db}$) had the highest increase in zinc content suggesting that most of the zinc-requiring fermentation microorganisms associated with the fresh leaves may have been eliminated during drying. RC$_{db}$ had significantly ($P < 0.05$) higher amounts of zinc released (0.021 g kg^{-1}) reflecting a 92.0% increase over the control. The increase in the zinc content of RC$_{db}$ (0.0053 g kg^{-1}) over the dried roselle calyces (RC$_{d}$) could also be due to fermentation-induced phytate reduction. Ojokoh *et al.*[28] reported a zinc content of 0.319 g kg^{-1} during fermentation of roselle calyces dried at 50 °C while Nnam and Onyeke[27] reported a lower value of 0.012 g kg^{-1}.

Vitamin A content of processed red palm oil

The vitamin A content of red palm oil emulsified with *B. eurycoma* or potash (Table 3) indicates that emulsification of red palm oil followed by drying led to reduced vitamin A contents; the amount of vitamin A lost was more in palm oil emulsified with *B. eurycoma* than potash (8.22 gRE kg^{-1} vs 4.84 gRE kg^{-1}) suggesting that *B. eurycoma* more effectively sequesters or destroys vitamin A and/or β-carotene compared to potash. Drying at 50 °C contributed to reduced vitamin A content as had been reported by Erhardt,[29] and Uzoma *et al.*[30] The results suggest that (1) the emulsifiers bind vitamin A and/or β-carotene, and (2) drying at 50 °C results in a loss of vitamin A and that potash induced a greater loss of vitamin A on drying after fermentation. The fermented *B. eurycoma*-palm oil emulsion dried at 50 °C had the highest release of vitamin A equivalent suggesting that the *B. eurycoma* system may have more effective buffering system resulting in lower acid production. Isomerization of trans-carotenoids to the less active cis-isomer during drying is promoted by contact with acids,[31] which could have happened when the fermented palm oil emulsions were dried. The heat of drying at 50 °C may possibly contribute to the loss of the carotenoids through accelerated oxidation to

Table 2. Iron and zinc contents of processed roselle calyces

Treatment	*Iron content (g kg^{-1})	Difference from control (g kg^{-1})	*Zinc content (g kg^{-1})	Difference from control (g kg^{-1})
C	$0.017^{a} \pm 0.004$	0	$0.022^{a} \pm 0.100$	0
RC$_{d}$	$0.052^{c} \pm 0.006$	0.035	$0.037^{c} \pm 0.010$	0.015
RC$_{b}$	$0.039^{b} \pm 0.093$	0.023	$0.034^{b} \pm 0.030$	0.012
RC$_{db}$	$0.056^{d} \pm 0.056$	0.039	$0.043^{d} \pm 0.030$	0.021

* Results are the means of two replications.
Values carrying different superscripts in the same column are significantly different ($P < 0.05$).
C = fresh roselle calyces (control); RC$_{d}$ = roselle calyces dried at 50 °C; RC$_{b}$ = roselle calyces fermented by backslopping; RC$_{db}$ = roselle calyces dried at 50 °C and fermented by backslopping.

Table 3. Vitamin A content (µg RE/kg) of palm oil emulsified with *B. eurycoma* and potash

	Vitamin A content			
Treatment	**B. eurycoma*	*Vitamin A released	*Potash	*Vitamin A released
C	$26210^c \pm 0.007$	0	$26210^c \pm 0.007$	0
E_d	$17990^a \pm 0.018$	−8220	$21370^a \pm 0.088$	−4840
E_f	$27480^d \pm 0.230$	1270	$33430^d \pm 0.014$	7220
E_{fd}	$24170^b \pm 0.381$	−2040	$21750^b \pm 0.007$	−4460

* Results are the means of two replications.
Values carrying different superscripts in the same column are significantly different ($P < 0.05$).
C = unemulsified red palm oil (control); E_d = red palm oil emulsified and dried at 50 °C; E_f = red palm oil emulsified and fermented by backslopping; E_{fd} = red palm oil emulsified, fermented by backslopping and dried at 50 °C.

products, which usually break down to low molecular mass compounds.[31]

These results suggest that addition of the processed cattle bones, roselle calyces and red palm oil may be best if added pre-fermentation.

Anti-nutrients content of the maize-bambara groundnut (*70 : 30*) mix

The anti-nutrient (tannin, oxalate and phytate) contents of the maize–bambara groundnut blends are presented in Table 4. Tannin contents decreased as a result of dehulling of bambara groundnuts, degerming of maize, malting of bambara groundnut and the fermentation of the blends. The reduction ranged from 25% to 62.5%, a very significant ($P < 0.05$) decrease compared to the control (MB_u); malting is known to increase polyphenol contents,[32] but fermentation reduces them.[4] The reduction in tannin content of the maize–bambara groundnut malt (MB_m) therefore, suggests some loss during soaking with little synthesised during germination. Moreover, since the white bambara groundnut grains were used, it does suggest that tannins synthesised may have been used for other metabolic functions. The highest decrease in tannin content (62.5%) was observed in the fermented maize-bambara groundnut malt [$(MB_m)_f$] and could be attributed to the activity of malt enzymes. Similar results were reported by Obizoba and Egbuna.[33]

Table 4. Anti-nutrient composition of maize–bambara groundnut complementary foods

Sample	*Tannin (g/kg)	*Oxalate (g/kg)	*Phytate (g/kg)
B_u	$0.004^e \pm 0.03$	$0.0008^d \pm 0.01$	$0.0022^e \pm 0.01$
MB	$0.003^d \pm 0.01$	$0.0003^b \pm 0.00$	$0.0018^d \pm 0.01$
MB_m	$0.002^b \pm 0.02$	$0.0003^b \pm 0.00$	$0.0014^b \pm 0.00$
$(MB)_f$	$0.0023^c \pm 0.01$	$0.0004^c \pm 0.01$	$0.0016^c \pm 0.02$
$(MB_m)_f$	$0.0015^a \pm 0.01$	$0.0002^a \pm 0.00$	$0.001^a \pm 0.01$

* Results are the means of three replications.
Values carrying different superscripts in the same column are significantly different ($P < 0.05$). Mb_u = Untreated maize-bambara groundnut blends; MB = maize–wet dehulled bambara groundnut; MB_m = maize–bambara groundnut malt; $(MB)_f$ = maize–bambara groundnut fermented by backslopping; $(MB_m)_f$ = maize–bambara groundnut fermented by backslopping.

The results for phytate and oxalate contents followed a similar trend as the tannins. The phytate composition of the blends was generally low and could be due to the effect of malting and germination.[9,34,35] The 50–75% decrease in oxalate contents of the blends could be attributed to the effect of steeping and fermentation.[36,37]

The tannin, oxalate and phytate levels observed were lower than the safe levels of 20 g kg^{-1}, 22 g kg^{-1} and 50 g kg^{-1}, respectively,[39] and could ensure the availability of divalent cations (calcium, iron and zinc) in the product and that no adverse physiological effects are produced when the products are consumed.

Composition of fortified maize-bambara groundnut complementary blends

The results (Table 5) show the *proximate composition* of fortified maize–bambara groundnut complementary food blends. The moisture content of the samples was low (40–50.4 g kg^{-1}) and could be due to loss of water holding capacity as a result of hydrolysis of macromolecules such as starch and protein during malting and fermentation.

The crude protein contents of the fortified samples varied from 180 to 190.5 g kg^{-1}. $(MB)_b$ and $(MB_m)_b$ showed increases of 0.54% and 1.6%, respectively, which could be attributed to microbial biomass and additional contributions from the protein content of *B. eurycoma*. $(MB)_t$ and $(MB_m)_t$ had lower protein contents compared to the controls suggesting that potash has a negative effect on protein content.

The fat content of the fortified blends ranged from 119.5 to 123 g kg^{-1} and were significantly ($P < 0.05$) higher than the unfortified blends. The high values could be due to contributions from red palm oil and could improve vitamin A absorption from the food.

The carbohydrate content of fortified blends varied from 595 to 604 g kg^{-1}. The values were 8.20–8.95% lower than unfortified blends and could be attributed to the relative increases in protein, fat, crude fibre and ash contents. The higher carbohydrate contents of samples containing red palm oil emulsified with *B. eurycoma* could be due to contributions from its carbohydrate content.

The fibre content of the fortified blends ranged from 20 to 21.5 g kg^{-1} and does not reflect any significant changes compared to unfortified blends. The low fibre content may be due to the use of dehulled raw materials in the formulation.

The ash content of the fortified blends (28–30.5 g kg^{-1}) were 43.58–46.70% higher than the unfortified blends. This increase might be attributed to the effect of fortification and loss of organic matter during fermentation. Obizoba and Atti,[39] observed increases in ash content in fermented unsprouted and sprouted sorghum seeds. The higher ash content in samples containing potash could be due to its inorganic matter content.

Calcium, iron, zinc and vitamin A contents

The calcium, iron, zinc and vitamin A contents of the fortified maize–bambara groundnut blends are shown in Table 6. The calcium content of the fortified blends ranged from 3.26 to 4.23 g kg^{-1}; the increase over the unfortified samples was some 150–230% and could be attributed to the high calcium content of cattle bone and the effects of ashing and fermentation. The calcium content of the fortified malt blends [$(MB_m)_b$

Table 5. Proximate composition of the maize–bambara groundnut blends

	Samples					
Proximate content (g/kg)	$(MB)_f$*	$(MB_m)_f$*	$(MB)_b$*	$(MB_m)_b$*	$(MB)_t$*	$(MB_m)_t$*
Moisture	$47.5^{c} \pm 0.01$	$45.0^{b} \pm 0.01$	$44^{b} \pm 0.05$	$40^{a} \pm 0.05$	$50^{d} \pm 0.02$	$40^{a} \pm 0.04$
Crude protein	$183.5^{b} \pm 0.05$	$187.5^{d} \pm 0.25$	$184.5^{c} \pm 0.02$	$190.5^{e} \pm 0.04$	$180^{a} \pm 0.02$	$184.5^{c} \pm 0.26$
Crude fat	$42.5^{b} \pm 0.04$	$40^{a} \pm 0.02$	$119.5^{c} \pm 1.76$	$123^{d} \pm 0.70$	$120^{c} \pm 0.04$	$122.5^{d} \pm 0.03$
Carbohydrate	$686^{e} \pm 0.50$	$684.5^{d} \pm 1.25$	$604^{c} \pm 1.15$	$595^{a} \pm 0.55$	$602^{b} \pm 0.06$	$602.5^{b} \pm 0.02$
Crude fibre	$20^{a} \pm 0.02$	$20^{a} \pm 0.02$	$20^{a} \pm 0.04$	$21.5^{b} \pm 0.10$	$20^{a} \pm 0.01$	$20^{a} \pm 0.05$
Ash	$19.5^{a} \pm 0.03$	$20^{a} \pm 0.03$	$28^{b} \pm 0.02$	$30^{c} \pm 0.25$	$28^{b} \pm 0.03$	$30.5^{c} \pm 0.03$

* Results are the means of three replications.

Values carrying different superscripts in the same row are significantly different ($P < 0.05$).

$(MB)_f$ = maize–bambara groundnut fermented by backslopping; $(MB_m)_f$ = maize–bambara groundnut malt fermented by backslopping; $(MB)_b$ = maize–bambara groundnut enriched pre-fermentation with fortificants containing *B. eurycoma* emulsified palm oil $(MB_m)_b$ = maize–bambara groundnut malt enriched pre-fermentation with fortificants containing *B. eurycoma* emulsified palm oil $(MB)_t$ = maize–bambara groundnut enriched pre-fermentation with fortificants containing potash emulsified palm oil; $(MB_m)_t$ = maize–bambara groundnut malt enriched pre-fermentation with fortificants containing potash emulsified palm oil.

Table 6. Calcium, iron, zinc (g/kg) and vitamin A (μg RE/kg) *content* of fortified maize–bambara groundnut food blends

	Micronutrient concentration			
Sample	Calcium*	Iron*	Zinc*	Vitamin A*
$(MB)_f$	$1.24^{a} \pm 0.02$	$0.027^{a} \pm 0.01$	$0.003^{a} \pm 0.02$	$330.1^{a} \pm 0.02$
$(MB_m)_f$	$1.27^{b} \pm 0.02$	$0.028^{b} \pm 0.02$	$0.004^{a} \pm 0.00$	$411.7^{b} \pm 0.04$
$(MB)_b$	$4.03^{f} \pm 0.01$	$0.084^{f} \pm 1.40$	$0.015^{c} \pm 0.00$	$5046.1^{d} \pm 0.06$
$(MB_m)_b$	$4.23^{g} \pm 0.02$	$0.134^{g} \pm 0.28$	$0.015^{d} \pm 0.39$	$7493.7^{f} \pm 0.05$
$(MB)_t$	$3.26^{c} \pm 0.05$	$0.075^{d} \pm 0.00$	$0.016^{e} \pm 0.00$	$4855.3^{c} \pm 1.96$
$(MB_m)_t$	$3.39^{d} \pm 0.70$	$0.083^{e} \pm 0.00$	$0.017^{f} \pm 1.73$	$5858.2^{e} \pm 0.02$
Nutrend	3.89	0.11	0.071	ND
Nutrend (nutritional label info.)	$3.90^{e} \pm 0.03$	$0.10^{c} \pm 0.05$	$0.07^{b} \pm 0.20$	$14940^{g} \pm 0.07$
RD**A**	5	0.275	0.125	5000

Results are the means of three replications.

* Values carrying different superscripts in the same column are significantly different ($P < 0.05$).

$(MB)_f$ = maize–bambara groundnut fermented by backslopping; $(MB_m)_f$ = maize–bambara groundnut malt fermented by backslopping; $(MB)_b$ = maize–bambara groundnut fortified with bone, roselle calyces and red palm oil emulsified with *B. eurycoma;* $(MB_m)_b$ = maize–bambara groundnut malt fortified with processed cattle bone, roselle calyces and red palm oil emulsified with *B. eurycoma;* $(MB)_t$ = maize–bambara groundnut fortified with processed cattle bone, roselle calyces, and red palm oil emulsified with potash; $(MB_m)_t$ = maize–bambara groundnut malt fortified with processed cattle bone, roselle calyces, and red palm oil emulsified with potash. ND = not determined; RDA = recommended daily allowance.

and $(MB_m)_t$] were significantly higher than their corresponding unfortified and their unmalted blends and may be due to the reduction of tannin, phytate and oxalate concentrations,[25,34] during germination of bambara groundnut and fermentation of the maize–bambara groundnut blends. Samples containing red palm oil emulsified with potash had significantly ($P < 0.05$) lower calcium contents compared to samples with *B. eurycoma*-emulsified palm oil and could be ascribed to a longer lag time in the activity of the fermentation microflora and a lower rate of hydrolysis of calcium–phytate complexes in potash samples. Samples containing red palm oil emulsified with *B. eurycoma* had significantly ($P < 0.05$) higher calcium content than Nutrend.

The increase in non-heme iron content after fortification with processed roselle calyces varied from 180.75% to 385.47% and were highly significant ($P < 0.05$). The increase could be due to phytate complex degradation by phytases during germination and fermentation.[25,40] Samples containing red palm oil emulsified with *B. eurycoma* [$(MB)_b$ and $(MB_m)_b$] had iron contents significantly ($P < 0.05$) higher than their corresponding potash counterparts. Only the fortified malt blends containing red palm oil emulsified with *B. eurycoma* [$(MB_m)_b$] contained $0.034\ \mathrm{g\ kg^{-1}}$ higher iron content compared to Nutrend ($0.11\ \mathrm{g\ kg^{-1}}$). The difference in iron content could be attributed to: (1) the possible influence of potash in inhibiting the hydrolysis of iron from organic complexes during fermentation; and (2) tying up of iron by potash.

The increase in zinc content of the fortified complementary food blends followed the same trend as iron. The increases above the unfortified blends varied from 0.012 to $0.013\ \mathrm{g\ kg^{-1}}$ and could be attributed to the zinc content of fresh roselle calyces (0.022 g/kg; Table 2) used in fortification and the activities of phytase. The higher amount of zinc in the potash sample may imply that the amount of potash used was not enough to bind the zinc content since zinc is less reactive than calcium and iron, and probably so compared to vitamin A. It may also reflect contributions from zinc impurities in potash. $(MB_m)_t$ had the highest zinc content which was significantly lower than Nutrend.

The vitamin A content of fortified maize–bambara groundnut complementary foods were significantly ($P < 0.05$) higher than the unfortified with values ranging from 4525.2 to 7082 μgRE kg^{-1} and could be due to the high retinol equivalent of the palm oil or the high zinc content,[41] of the blends. It may also be due to the emulsifiers acting as stabilisers for the retinol formed,[42] by delaying or inhibiting oxidation of vitamin A. Samples containing red palm oil emulsified with *B. eurycoma* had significantly ($P < 0.05$) higher vitamin A contents than their corresponding potash counterparts probably resulting from the additive effect of *B. eurycoma* vitamin A[43] or from the promotional effect of *B. eurycoma* protein in the conversion of carotenoids to vitamin A.[44] The vitamin A contents of all the maize–bambara groundnut blends were lower than that of Nutrend and could be attributed to loss of vitamin A during acid fermentation,[31] drying at 50 °C and the fact that the conversion efficiency of β-carotene to vitamin A is only about 60%.[45] The use of these emulsifiers/stabilisers, particularly potash, in traditional foods may be suspect except that subsequent cooking in the case of soup making with *B. eurycoma* may lead to the release of vitamin A or that enzymes in the gastrointestinal tract may release vitamin A from the *B. eurycoma* and potash-treated foods.

CONCLUSION

This study has shown that pre-fermentation fortification of maize–bambara groundnut complementary foods for infants can be achieved with *B. eurycoma* or potash-emulsified red palm oil mixed with processed cattle bone and *H. sabdariffa*. Malted bambara groundnut blends containing red palm oil emulsified with *B. eurycoma* $[(MB_m)_b]$ had higher protein, calcium, iron and vitamin A contents when compared with potash samples.

The products formulated during this investigation were produced from crops that are produced in commercial quantities by the rural poor, mostly in the northern parts of Nigeria. The technologies are largely household level which are easily accessible to the rural and urban poor without the need for expensive equipment. They can more easily access these in order to produce these enriched complementary foods for infants in 12 days using the heat of the sun, which is available most of the year for drying.

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