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EVALUATION OF THE VITAMINS, MINERALS, AND PHYTOCHEMICAL CONTENTS OF COMPLEMENTARY FOOD BLENDS FROM PEARL MILLET (PENNISETUM GLAUCUM), AFRICAN YAM BEAN (SPHENOSTYLIS STENOCARPA HOECHST EX. A. RICH), AND TIGER NUT (CYPERUS ESCULENTUS).

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Improvement and use of complementary food blends is an economic option in an attempt to reduce the risk of chronic diseases usually associated with malnutrition. Ingredients utilized in complementary food blends are normal foods composed of high levels of one or more essential nutrients and low levels of anti-nutrients and are available at comparatively lower cost and underutilized. The aim of this study was to produce complementary food blends from pearl millet, African yam bean and tiger nut and evaluate the vitamin, mineral and anti-nutrient contents. Ten complementary food blends were formulated and the vitamins, minerals and anti-nutrients were evaluated. The data obtained were statistically analyzed using Analysis of Variance (ANOVA) technique with the use of Statistical Package for Social Sciences (SPSS) version 21.0. Means were compared and separated using Duncan's Multiple Range Test (DMRT) and LSD at $p \leq 0.05$. The results obtained showed that vitamin A ranged from 0.32 – 0.76 mg/ 100g; vitamin B1, 0.13–0.14 mg/ 100g; vitamin B3, 0.12 –0.17 mg/ 100g. Results indicated that calcium contents ranged from 10.00–54.00 mg/ 100g; magnesium 10.50–11.40 mg/ 100g; potassium 25.90–27.00 mg/ 100g' iron 7.30–9.50 mg/ 100g and phosphorus 20.40–26.60 mg/ 100g. For the anti-nutrients, tannin ranged from 32.00–39.00 mg/ 100g; phytate 22.00–37.00 mg/ 100g and oxalate 6.80–11.10 mg/ 100g. These anti-nutrients obtained in this study falls within the permissible limits of

Corresponding author* Obomeghei A. A.	20mg/g, 3–5 mg/kg and 200–250 mg/ g for tannin, phytate and oxalate respectively.

I INTRODUCTION

The consumption of cereal-based infant's complementary foods is very common and popular globally especially in developing countries where they constitute a major source of their staple foods. The rates of Nigeria's malnutrition and intense wasting are known to be very high at about 1.9 million infants and children yearly.[1] The effort to identify and include non-cereal, rarely used and cheap food ingredients with good nutrient density in the production of complementary food is therefore important.[2] Developing countries possess lots of nutrient dense, inexpensive and easily available plant food sources that can meet the nutritional requirements for infants and children when combined together in appropriate proportions.[3] These plant food sources naturally contain essential vitamins and minerals in addition to anti-nutrients. Anti-nutrients are usually eliminated or reduced while vitamins and minerals may increase during food processing. According to Igile *et al.* [4] there are strong evidence that some anti-nutrients like oxalates and cyanates could make foods more nutritious, but necessarily improve human health. Pearl millet has been utilized as a food crop for several years in many different food products and will continue to be used as staple grains by about 90 million persons in Africa and India. [5] Pearl millet (Pennisetum glaucum) is one of the most significant cereal crops cultivated in tropical regions and developing nations. [6] Millet grains have been reported to be nutritionally comparable and even superior to major cereals concerning protein, energy, vitamins and minerals. Besides, they are a rich source of dietary fiber, phytochemicals and micronutrients and hence they are rightly described as "Nutri cereals" [7] African yam bean is a legume that is rich in starch and protein and it constitutes an important dietary source of the mineral calcium and several amino acids. The seed and tuber of African yam bean are composed of different food components and minerals that are comparable to other legumes. African yam bean is rich in minerals such as potassium, phosphorous, magnesium, calcium, iron and zinc but low in sodium and copper. [8] Tiger nut has been considered a foodstuff since ancient times. [9] Tigernut (Cyperus esculentus) is an underutilized, inexpensive and readily available crop in Nigeria. The tubers contain a significant amount of protein, fat, minerals and vitamins. [10, 11] Tiger nut tubers contain digestive enzymes like catalase, lipase and amylase which assist to alleviate indigestion, flatulence and diarrhea. Its high oleic content helps to reduce cholesterol levels in the human body that prevents heart attacks and thrombosis. Tiger nut tubers are a good source of Vitamin B1. Regular consumption of the tubers improves men's and women's fertility due to their Vitamin E content. The Vitamin E present in the tubers is believed to delay aging in human cells, bring about improvement in the elasticity of the skin and get rid of wrinkles and undesirable changes that could affect the skin. [12]

The processing of cereals, roots, tubers and legumes into flour involves washing, soaking, dehulling, dehydration and milling. Losses in nutrients, especially vitamins and minerals, are expected in steeping, dehulling, dehydration, and milling. These unit operations affect food nutrients to different extents depending on the product being processed. [13] Olapade *et al.* [14] had evaluated the functional and pasting properties of complementary food blends from pearl millet, African yam bean, and tiger nut. And there is a need to establish the status of vitamins, minerals and anti-nutrients present in the blends to ensure adequacy of these vital nutrients in the infant weaning foods to be prepared from them. This research is therefore aimed at determining the Vitamins, minerals and anti-nutritive concentrations of pearl millet, African yam bean and tiger nut complementary food blends

II MATERIALS AND METHODS

Materials

African yam beans were obtained from Genetic Resources Centre, International Institute for Tropical Agriculture (IITA), Ibadan. While pearl millets and tiger nuts were purchased from a local dealer at Bodija market, Ibadan.

Methods

Production of millet flour

The malted millet flour was prepared according to the method of Elemo *et al.* [15] One kilogram (1kg) of millet grains were sorted to move dirt and other extraneous materials. The grains were thoroughly cleaned and steeped in 3.5 liters of potable water in a plastic bowl at room temperature (29±2°C) for 24 h with a change of water at every 8 h to prevent fermentation. The steeped grains were drained, rinsed and immersed in 2% sodium hypochlorite solution for 10min to sterilize the grains. The grains were rinsed five consecutive times with excess water and cast on a damped jute bag, covered with a polyethylene bag and left for 24 h to fasten sprouting. The grains were carefully spread on the jute bag and allowed to germinate in the germinating chamber at ambient temperature (29±2°C) and relative humidity of 95% for 120 h. During this period, the grains were sprinkled with water at intervals of 10 h to facilitate germination. Non- germinated grains were handpicked and discarded. The germinated grains were spread on the trays and dried in a cabinet dryer (Model HR 6200, UK) at 60°C for 20 h with occasional stirring of the grains at intervals of 30 min to ensure uniform drying. The dried malted grains were cleaned, rubbed in–between palms and winnowed to remove the roots and the sprouts. The millet malts were milled in an attrition mill (Franky DM-WP 200 Electric Cereal mill). The flours were packaged in high-density polyethylene (0.08mm thick), kept in a covered plastic bucket, and stored in a deep freezer (-10^{0}C)

Production of tiger nut flour

The method described by Adejuyitan. [16] was used to prepare tiger nut flour. Dry tiger nut was sorted to remove extraneous materials and washed with potable water. The clean nuts were dried at 105^{0}C for 3hrs. The dried nuts were milled in an attrition mill (Franky DM-WP 200 Electric Cereal mill), sieved and packaged in high-density polyethylene. These were kept in a covered plastic bucket and stored in a deep freezer (-10^{0}C).

Production of African yam beans flour

The method of Eke [17] was used for the preparation of African yam bean flour. The seeds were handpicked, carefully sorted and winnowed to remove immature and unwholesome seeds and other extraneous materials. The cleaned seeds were steeped in warm water (45^{0}C; 10hrs) in a thermostatic water bath. The seeds were manually dehulled and washed and then decanted to remove the seed coats. The seeds were then dried in a cabinet drier (60^{0}C; 8hrs) and milled in an attrition mill (Franky DM-WP 200 Electric Cereal mill) through a 210µm sieve and packaged in high-density polyethylene. These were packaged in a covered plastic bucket and stored in a deep freezer (-10^{0}C).

Determination of Vitamins

Determination of Vitamin A.

Vitamin A contents of the flour blends were determined using the method described by AOAC. [18] One gram (1 gm) of each sample was weighed into sample glass bottles. Ten (10) ml of n-hexane was added and shaken using a laboratory shaker for about 1hour. Samples were allowed to stay overnight in the dark. The mixtures were then filtered with Whatman No. 1 filter paper and left in the fume hood for the hexane to evaporate. 5ml of HPLC grade methanol was added and the sample was injected into HPLC and the reading was taken at 325 nm.

Formulation of complementary food blends

Ten (10) complementary food blends were formulated in the specified ratio to make 100% as shown in Table 1.

Table 1. Formulation of complementary food blends (%)

S/N	Sample code	PMF	AYBF	TNF
1	ITS	70	20	10
2	OTO	75	10	15
3	BSA	80	10	10
4	OAB	70	15	15
5	OAK	71.67	11.67	16.67
6	BUI	70	10	20
7	CUO	73.33	13.33	13.33
8	BEL	76.67	11.67	11.67
9	LAC	71.67	16.67	11.67
10	OPE	75	15	10

PMF = Pearl millet flour, AYBF = African yam bean flour, TNF = Tiger nut flour

Determination of thiamine (Vitamin B1)

Thiamine contents were determined using the scalar analyzer method as described by AOAC. [18] Five grams of sample was homogenized in 5 ml normal ethanoic NaOH solution. The homogenate was filtered and made up to 100 ml with the extract solution. Ten (10 ml) of an aliquot of the extract was dispersed into a flask and 10 ml of potassium dichromate solution was added. The resultant solution was incubated at room temperature for 15 minutes. The absorption was read from the spectrometer operating at 360nm using a reagent blank to standardized the instrument at zero.

Thiamine $\left(\frac{mg}{100g}\right) = \frac{100}{W} \times \frac{au}{as} \times C \times D$ … (1)

Where, au = Absorbance of the sample solution
as = Absorbance of the standard solution
C = Concentration of the standard solution
D = Dilution factor
W = Weight of the sample

Determination of niacin (Vitamin B3)

Samples (5 g) were treated with 50 ml of 1N sulphuric acid and shaken using a laboratory shaker for about 30 minutes. Three drops of ammonia solution were added to the samples before filtration. 10 ml of the filtrate was pipette into a 50 ml volumetric flask and added 5 g potassium cyanide. These were then acidified using 5 ml. of 0.02N H_2SO_4 and the absorbances measured in the spectrophotometer at 470 nm wavelength. [19]

Determination of minerals

The amounts of mineral elements present in the samples were determined using the method of Atomic Absorption Spectrophotometer (Jenway, Model 7315; Bibby Scientific Ltd, United Kingdom). And the phosphorus contents were determined using the spectrophotometric method that is based on the measurement of absorbances of the yellow color obtained by the reaction of the element with molybdate vandate solution. [20]

Determination of anti-nutrients

The amounts of phytate and oxalate present in the samples were determined as described by AOAC. [21] while the phosphorus contents were determined as described by the method of Medoua *et al.* [22]

Statistical Analysis

The data obtained were statistically analyzed using Analysis of Variance (ANOVA) technique with the use of Statistical Package for Social Sciences (SPSS) version 21.0. Means were compared and separated using Duncan's Multiple Range Test (DMRT) and LSD at p≤0.05.

III RESULTS

Table 2 shows the data obtained for the vitamin contents of formulated complementary food blends. The vitamin A content ranged from 0.32 to 0.76 mg/ 100g. The vitamin B1 contents of the complementary flour blends ranged between 0.13 and 0.14 mg/100g. While the Vitamin B3 contents of the samples ranged from 0.12 to 0.17 mg/ 100g.

Table 2. Vitamin contents of complementary food blends from millet, African yam bean and tiger nut (mg/ 100g).

Samples	Vitamin A	Vitamin B1	Vitamin B3
ITS	$0.45{\pm}0.23^{cd}$	$0.14{\pm}0.00^{ab}$	$0.14{\pm}0.00^{cd}$
OTO	$0.32{\pm}0.12^{e}$	$0.13{\pm}0.00^{d}$	$0.12{\pm}0.00^{e}$
BSA	$0.38{\pm}0.14^{de}$	$0.13{\pm}0.00^{ce}$	$0.14{\pm}0.00^{cd}$
OAB	$0.45{\pm}0.17^{cd}$	$0.14{\pm}0.00^{ab}$	$0.16{\pm}0.00^{b}$
OAK	$0.72{\pm}0.11^{ab}$	$0.13{\pm}0.00^{d}$	$0.15{\pm}0.00^{b}$
BUI	$0.52{\pm}0.04^{cd}$	$0.14{\pm}0.00^{ab}$	$0.12{\pm}0.00^{d}$
CUO	$0.76{\pm}0.39^{a}$	$0.13{\pm}0.00^{cd}$	$0.14{\pm}0.00^{c}$
BEL	$0.43{\pm}0.03^{cd}$	$0.14{\pm}0.00^{ab}$	$0.11{\pm}0.00^{f}$
LAC	$0.48{\pm}0.02^{cd}$	$0.14{\pm}0.00^{abc}$	$0.17{\pm}0.00^{a}$
OPE	$0.46{\pm}0.22^{cd}$	$0.14{\pm}0.00^{abc}$	$0.17{\pm}0.00^{a}$

Values within a column with different superscript are significantly different (p≤0.05)

The mineral contents of the samples of complementary food blends are presented in Table 3. The calcium contents ranged from 10.00 to 54.00 mg/ 100g. The magnesium contents ranged between 10.50 and 11.40 mg/ 100g. The potassium contents of the flour blends ranged from 25.90 to 27.00 mg/ 100g. The iron contents of the complementary flour blends ranged between 7.30 mg/ 100g and 9.50 mg/ 100g. While the values of phosphorus ranged between 20.40 and 26.60 mg/ 100g.

Table 3. Mineral contents of complementary flour blends from millet, African yam bean and tiger nut (mg/ 100g).

Samples	Calcium	Magnesium	Potassium	Iron	Phosphorus
ITS	$10.00{\pm}0.00^{c}$	$11.00{\pm}0.00^{c}$	$26.70{\pm}0.00^{b}$	$7.30{\pm}0.00^{c}$	$20.40{\pm}0.00^{h}$
OTO	$10.0{\pm}0.00^{c}$	$11.00{\pm}0.00^{c}$	$27.00{\pm}0.00^{a}$	$9.50{\pm}0.00^{a}$	$22.50{\pm}0.00^{d}$
BSA	$10.00{\pm}0.00^{c}$	$11.40{\pm}0.00^{a}$	$25.90{\pm}0.00^{f}$	$8.90{\pm}0.00^{c}$	$23.40{\pm}0.00^{c}$
OAB	$10.00{\pm}0.00^{c}$	$10.60{\pm}0.00^{d}$	$26.10{\pm}0.00^{e}$	$8.80{\pm}0.00^{d}$	$26.40{\pm}0.00^{b}$
OAK	$10.00{\pm}0.00^{c}$	$10.50{\pm}0.00^{d}$	$26.50{\pm}0.00^{c}$	$8.10{\pm}0.00^{g}$	$21.70{\pm}0.00^{f}$
BUI	$10.00{\pm}0.00^{c}$	$11.20{\pm}0.00^{b}$	$26.30{\pm}0.00^{d}$	$9.50{\pm}0.00^{a}$	$21.40{\pm}0.00^{g}$
CUO	$54.00{\pm}0.06^{b}$	$10.60{\pm}0.00^{d}$	$26.40{\pm}0.00^{d}$	$9.10{\pm}0.00^{b}$	$26.60{\pm}0.00^{a}$
BEL	$11.00{\pm}0.00^{c}$	$11.10{\pm}0.00^{c}$	$26.90{\pm}0.00^{a}$	$8.40{\pm}0.00^{e}$	$22.40{\pm}0.00^{c}$
LAC	$1.20{\pm}0.00^{c}$	$10.50{\pm}0.00^{d}$	$26.40{\pm}0.00^{d}$	$8.30{\pm}0.00^{f}$	$22.20{\pm}0.00^{e}$
OPE	$9.00{\pm}0.00^{a}$	$10.50{\pm}0.00^{d}$	$26.00{\pm}0.00^{g}$	$8.10{\pm}0.00^{f}$	$20.40{\pm}0.00^{h}$

Values within a column with different superscript are significantly different (p≤0.05)

The anti-nutrient contents of the complementary flour blends are shown in Table 4. The tannin contents ranged between 32.00 and 39.00 mg/ 100g. The phytate contents of complementary food blends obtained in this experiment were between 22.10 and 37.00 mg/ 100g. While the values of oxalate obtained for complementary food blends in this study ranged from 6.80 to 11.10 mg/ 100g.

Table 4. Anti-nutrient contents of complementary food blends from millet, African yam bean and tiger nut flours (mg/ 100g)

Samples	Tannin	Phytate	Oxalate
ITS	$31.80{\pm}0.02^{d}$	$28.60{\pm}0.01^{d}$	$10.20{\pm}0.00^{b}$
OTO	$39.20{\pm}0.03^{a}$	$32.80{\pm}0.01^{bc}$	$8.90{\pm}0.00^{c}$
BSA	$37.60{\pm}0.01^{ab}$	$22.10{\pm}0.00^{f}$	$8.40{\pm}0.00^{c}$
OAB	$33.50{\pm}0.00^{cd}$	$29.40{\pm}0.00^{d}$	$8.80{\pm}0.00^{c}$
OAK	$35.20{\pm}0.00^{bc}$	$29.50{\pm}0.01^{d}$	$6.80{\pm}0.00^{d}$
BUI	$32.40{\pm}0.00^{cd}$	$37.00{\pm}0.01^{a}$	$8.50{\pm}0.00^{c}$
CUO	$32.50{\pm}0.00^{cd}$	$25.40{\pm}0.00^{e}$	$8.00{\pm}0.00^{c}$
BEL	$32.50{\pm}0.00^{cd}$	$31.70{\pm}0.01^{c}$	$11.10{\pm}0.00^{a}$
LAC	$32.90{\pm}0.02^{cd}$	$28.90{\pm}0.02^{d}$	$10.30{\pm}0.02^{ab}$
OPE	$36.40{\pm}0.00^{ab}$	$34.30{\pm}0.00^{b}$	$8.30{\pm}0.00^{c}$

Values within a column with different superscript are significantly different (p≤0.05)

IV DISCUSSION

There were significant differences (p≤0.05) in vitamin A contents of the samples. Sample CUO is significantly higher in vitamin A content than all other samples. Samples OAK and CUO are not significantly different from each other. Also samples ITS, OAB, BUI, BEL, LAC, and OPE are not significantly different from each other in terms of vitamin A. Sample OTO is significantly lower than all other samples.

There was no significant difference (p≤0.05) in vitamin B1 among samples ITS, OAB, BUI, BEL, LAC and OPE. But these were significantly higher than samples OTO, BSA, OAK, and CUO.

There were significant differences (p≤0.05) among the samples in terms of vitamin B3. Samples LAC and OPE are not significantly different from each other but these two are significantly higher than all other samples. Samples BEL and BUI are significantly lower than all other samples.

The calcium contents were found to be significantly different (p≤0.05). Sample OPE is significantly higher than all other samples, followed by sample CUO. There were no significant differences among all other samples. Okoye *et al.* [23] obtained a range between 10.30 and 13.27 mg/ 100g (which is within the range obtained in this study) for loaves of bread produced from Millet-African-Bread Fruit composite flour while Mbaeyi-Nwaoha and Obetta [24] obtained the range 2.00 to 6.05 mg/ 100g, which is far lower than the range obtained in this experiment. Calcium is important for bone and teeth development in infants and children along with the maintenance of healthy nerves and muscles. [2]

Significant differences (p≤0.05) existed in terms of magnesium among the samples. Sample BSA was found to be significantly higher than all other samples followed by sample BUI. There were no significant differences among samples ITS, OTO, and BEL. Also significant differences do not exist among samples OAB, OAK, CUO, LAC, and OPE. The magnesium contents obtained in this study are higher than 3.82 – 5.76 mg/ 100g reported by Okoye *et al.* [23]

Significant differences ($p \leq 0.05$) also existed among the samples in terms of potassium. Samples OTO and BEL are significantly higher than all other samples in terms of potassium contents. The potassium contents obtained in this study are higher than the values (5.09 – 7.57 mg/ 100g) reported by Okoye *et al.* [23] Potassium was found to be the most abundant mineral element present in these complementary flour blends.

The values of iron for samples OTO and BUI were found to be significantly higher than all other samples. The value for sample ITS is significantly lower than all other samples. The range reported in this study was found to be within the range (4.80 – 9.98 mg/ 100g) reported for breadfruit-soybean complementary flour blends[2] but lower than the recommended dietary iron requirement (11.00 mg) for infants of age 7 – 12 months.[25] Mengistu *et al.*[26] reported a range of 2.52 – 5.10 mg/ 100g for complementary diets made from fermented cereals and soybean while Okoye *et al.*[23] reported a range 3.97 – 6.11 mg/ 100g. Iron is an important component of hemoglobin which is needed for the proper growth and formation of healthy blood cells.

Phosphorus is the next most abundant element present in the complementary flour blends in this study after potassium. Sample CUO is significantly higher in phosphorus than all other samples while the values of samples ITS and OPE are significantly lower than all other samples. The values of phosphorus obtained in this experiment were found to be higher than the values (4.53 – 6.39 mg/ 100g) reported by Okoye *et al.* [23]

There were significant differences ($p \leq 0.05$) in the tannin, phytate, and oxalate contents of all the complementary flour blends. There were no significant differences in tannin contents among samples OTO, BSA, and OPE. The values of these samples were significantly higher than the values for all other samples. Significant differences do not exist in the values of tannin among samples ITS, OAB, OAK, BUI, CUO, BEL, and LAC. Sample OTO had the highest value of tannin while sample ITS had the lowest value. The low levels of tannin contents could be attributed to the loss of tannin during soaking as a result of the leaching of tannins into the soak water. The values of tannin contents obtained in this study were slightly higher than the values 21.08 – 33.09 mg/ 100g reported for orange-fleshed sweet potato supplemented with cowpea and groundnut flour complementary food blends. [27] Ekwere *et al.* [28] reported lower values of 14.31 – 15.20 mg/ 100g. The total acceptable daily intake limit of tannin is 560 mg. [29]

Sample BSA is significantly ($p \leq 0.05$) lower in phytate than all other samples while the value for sample BUI is significantly higher than the values for all other samples. The values of phytates obtained in this study were found to be far lower than the range 42.56 – 61.06 mg/ 100g reported for orange-fleshed sweet potato supplemented with cowpea and groundnut flour complementary food blends. [27] and also far lower the value 229.85 mg/ 100g reported for orange-fleshed sweet potato-soybean-anchovy powder. [30] Mengistu *et al.* [26] reported higher values of 173 – 362 mg/ 100g for food blends made from fermented cereals and soybean.

There was no significant difference between the values of oxalate obtained for samples BEL and LAC but the values for these two were significantly different from the values for all other samples. Sample OAK had the least value while sample BEL had the highest value. The oxalate contents obtained in this study are higher than 3.94 – 6.19 mg/ 100g reported for orange-fleshed sweet potato supplemented with cowpea and groundnut flour complementary food blends.[27] The lower range of 0.55 – 0.82 mg/ 100g was reported for infant foods made from African yam bean and Bambara groundnut.[28] The oxalate contents obtained in this study are very low compared to the maximum recommended daily intake of 40 – 50 mg/ day.[31] Oxalates are usually regarded as undesirable constituents in food as a result of their capacity to reduce assimilation of calcium by favoring the formation of renal calcium. Oxalates and phytates do combine with calcium and phosphorus to form complexes and render them unavailable for absorption. [32]

The presence of these anti-nutrients in the complementary food blends in this study can be attributed to the high levels of tannins, phytates and oxalates that have been reported to be high in African yam bean. [33] However, these antinutrients obtained in this study fall within the permissible limits of 20mg/ g, 3 – 5 mg/ kg and 200 – 250 mg/ g for tannin, phytate and oxalate respectively.

V CONCLUSION

The formulated complementary food blends have been found to contain appreciable amounts of vitamin A, vitamin B1 and vitamin B3. The food blends were found to also contain good amounts of minerals such as calcium, magnesium, potassium and phosphorus and low levels of the anti-nutrients tannin, phytate and oxalate and therefore may not elicit toxicity reactions in infants and children and they are within acceptable levels.

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