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Micronutrient Composition and its Bio-availability in Complementary Foods Developed From Cereal (Millet/Maize), Soybean and Monkey kola Flours

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ABSTRACT

The micronutrient composition of complementary foods produced from blends of cereal (millet/maize), soybean and monkey kola flours were evaluated. Seven millet-based blends (A1 to G1) and maize-based blends (A2 to G2) were analyzed for total carotene content. Thereafter, 100% millet, 100% maize, the two millet and maize based blends that had the highest carotene content were analyzed for total minerals (Ca, Mg, P, Fe, and Zn) and their bio-availability comparing with a commercially available complementary product (cerelac maize) which served as control. The total carotene content of the test samples ranged from 27.69 to 164.58µg/100g in the millet-based blends and from 233.61 to 464.48µg/100g in the maize-based blends. Sample G1 and all the maize-based blends were found to be higher in total carotene when compared to the control. Total mineral content result showed that calcium ranged from 91.09 to 121.59mg/100g and their bioavailability ranged from 44.14 to 67.96% while the control had a total calcium content of 337.15mg/100g and a bio-availability of 58.92%. Magnesium in the test samples ranged from 10.44 to 12.29mg/100g and bio-availability of 82.56 to 99.33% while the control was found to be 11.18mg/100g and a bio-availability of 87.65%. Phosphorous was from 7.32 to 17.12mg/100g and bio-availability was from 54.48 to 81.43% but the control had 17.12mg/100g and a bio-availability of 61.35%. Iron had a range of 9.31 to 26.27mg/100g and bio-availability from 8.19 to 64.81%, whereas the control had 27.74mg/100g and a bio-availability of 51.47%. Zinc from 1.85 to 6.27mg/100g and bio-availability of 51.62 to 74.71% while C3 had 3.93mg/100g and a bio-availability of 42.86%. This means that complementary foods from blends of cereal, soybean and monkey kola flours compared with commercially available complementary products and are suitable to improve the micronutrient intake of infant and young children in developing countries.

Keywords: Complementary foods, micronutrient, cereal, millet, maize, monkey kola.

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1. Introduction

Micronutrients play a major role in the health and proper development of a child and lack of it in a child's diet results in serious health disorder. Calcium for instance is required for strong teeth formation in children and to prevent rickets. Rickets weakens the bones and results in bow legs, stunted growth and makes the muscles sore or weak. Lack of magnesium causes hormonal imbalance, lack of iron results in anaemia while vitamin A is responsible for proper eye sight and to prevent frequent ill health. Malnourished children often have low resistance to infections and diseases and so have high morbidity. UNICEF Nigeria (2006), reported that Nigeria and 9 other countries have the largest number of underweight children in the world with approximately 6 million underweight children who are under five years old and that micronutrient deficiency is directly responsible for child morbidity, in addition to food insecurity and improper feeding practices.

Previously, nutritionists had focused on improving child nutrition through inclusion of legumes to locally made complementary foods. Soybean has been found to be an excellent source of essential amino acid, being high in lysine and tryptophan. However, from the late 80s, nutritionists and food experts became more concerned about malnutrition resulting from micronutrient deficiencies in infants particularly vitamin A and mineral deficiency (Solomon 2000). Fruits and vegetables have been recommended as good sources of micronutrients according to FMOH (1999). Increase in minerals and vitamin A contents of complementary foods supplemented with fruits or vegetables have been encouraged (Onabanjo *et al*., 2009).

Monkey kola is a group of underutilized fruits found in the Southern part of Nigeria and Cameroon. They belong to the family *Malvaceae* and the varieties include *Cola parchycarpa* (yellow variety) *Cola lapidota* (white variety) and *Cola latarina* (red variety). *Cola parchycharpa* (yellow variety) was identified botanically in the Department of Forestry, Michael Opara University of Agriculture, Umudike, Abia State, Nigeria (Okudu *et al*., 2016). Kiin-Kabari *et al.* (2018) evaluated cereal-based complementary foods supplemented with soybean and monkey kola flours. Their results showed that all essential amino acids were present in the test samples with acceptable sensory characteristics and zinc. Eneobong *et al.* (2016) had also reported that monkey kola contains substantial amount of carotene and minerals such as calcium, magnesium, phosphorus, iron and zinc. However, information on the bio-availability of these minerals is scarce. Again, the pulp of *Cola parchycarpa* is hard; this limits its consumption among children and the aged with weak dentition. Therefore, it is necessary to incorporate monkey kola into a blend of cereal and soybean, to produce a balanced complementary food.

2. Materials and Method

Grains of millet (*Pennisetum glaucum*), maize (*Zea mays*), soybean (*Glycine max)* and fruits of yellow monkey kola (*Cola parchycarpa)* were bought from mile 3 market, Diobu Port Harcourt in Rivers State of Nigeria.

2.1. Processing of the Blends

Millet grains were sorted and cleaned to remove stones, sand, husks and grains of other cereals. Five kilogram of the cleaned millet was steeped in water for 48h, with the water changed every 24h. The steeped seeds were washed in clean water, boiled for 45min and dried in an oven temperature of 65^{o}C for 10h. The dried seeds were milled using an attrition mill to obtain millet flour. The flour was passed through a 150 micron particle size sieve to obtain the millet flour that was used for the analysis.

Maize grains were sorted, cleaned and 5kg of the cleaned grain was steeped in clean water for 48h with the water changed every 24h. After 48h, the seeds were washed, boiled for 45min and dried at an oven temperature of 65ºC for 10h. After drying, the seeds were milled using an attrition mill and the flour sieved using a 150 micron particle size sieve to obtain the maize flour which was used for the analysis.

Soybean seeds were sorted and cleaned; 5kg of the cleaned seeds was washed and steeped in clean water for 48h with the water changed every 24h. After 48h, the seeds were washed in clean water by abrasion between the palms to remove the seed coats, boiled for 45min, dried at 65ºC for 10h, milled and sieved using 150 micron particle size sieve to obtain soybean flour. The soybean flour so obtained was mixed with food grade hexane and allowed to stand for 1h. It was then poured into a muslin cloth and pressed using a screw press. The residue from the press was transferred into an oven tray and dried for 6h at a temperature of 70ºC to obtain the partially de-fated soybean flour that was used for the experiment.

Monkey kola (*Cola parchycarpa*) flour was prepared according to method of Okudu *et al*. (2016) with slight modification. After cleaning, the pulps were grated directly into a clean oven tray and dried for 10h at an oven temperature of 65ºC. The dried grated pulps were then milled using an attrition mill and sieved with a 150 micron size sieve. The resultant flour was stored in a deep freezer to be used for the analysis.

2.2. Formulation Ratios of the Complementary Blends

Different ratios of cereal (millet/maize) flours, soybean and monkey kola flours were formulated. The millet flour was supplemented with 0%, 10, 15, 20 and 25% each of soybean and monkey kola flours. Each combination was properly blended using a Philip rotary mixer (Type HR 1500A) to obtain a homogenous blend. The blends were packaged individually in air tight glass bottles and stored in a deep freezer for analysis. Seven other combinations of maize, soybean and monkey kola flours were formulated by supplementing the maize flour with 0, 10, 15, 20 and 25% each of soybean and monkey kola flours. Each combination was properly blended using a Philip rotary mixer (Type HR 1500A) to obtain a homogenous blend. The blends were packaged individually in air tight glass bottles and stored in a deep freezer for analysis. A commercial complementary product (cerelac maize) served as the control. The formulation for both the millet-based blends and the maize-based blends is shown in Table 1.

All test samples were analyzed for carotene content. Thereafter, 100% millet sample (A1), 100% maize sample (A2), two samples with higher carotene content in the millet-based blends (F1, G1) and two sample with higher carotene content in the maize based blends (F2, G2) were evaluated for total mineral content, soluble mineral content and their percentage mineral extractability calculated.

Table 1. Formulation of Millet-based and Maize-besed Complementary Blends

Sample codes		(%) Millet or maize	(%) Soybean	(%) *Cola Parchycharpa*
A1	A2	100	0	0
B1	B2	90	0	10
C1	C2	90	10	0
D1	D2	80	10	10
E1	E2	70	15	15
F1	F2	60	20	20
G1	G2	50	25	25

A1 – G1 = millet-based blends, A2 – G2 = maize-based blends.

2.3. Determination of Total Carotene Contents

The total carotene was determined using Harborne (1973) phytochemical methods. One hundred milligram of sample was weighed into a centrifuge tube and to it was added 10ml of 80% acetone. These were mixed thoroughly and centrifuged at 3000rpm for 10min. The mixture was then filtered and the supernatant made up to a volume of 10ml using 80% ethanol. The absorbance (optical density) was read at a wavelength of 480nm uv visible spectrophotometer. The total carotenoid content was then calculated as follows:

$$\text{Total carotenoid (mg/kg)} = 4x\frac{absorbance\ (optical\ density)\ x\ total\ sample\ volume}{Sample\ weight}\ x\ 1000$$

Total carotene (mg/kg) = Total carotenoid – Xanthophyll

Xanthophyll = 22% of total carotenoid

In µg/100g sample, Total carotene (mg/kg) = total carotene in 100µg/100g

2.4. Determination of Minerals

Calcium, magnesium, iron and zinc were determined by AOAC (2012) method, using Atomic Absorption Spectrophotometer (Perkin Elmer, 2380 USA). Phosphorus was determined by Molybdate method. However, only samples A1, A2, F1, F2 and G1, G2, judged to be acceptable were analysed for minerals (Kiin-Kabari *et al*., 2018).

2.5. Determination of Extractable Mineral Content of the Blends

Mineral extractability was done according to the Chauhan and Mahjan (1988) method. One gram sample was extracted using 10ml of 0.03N HCL with agitation at 37^{o}C for 3h. The extract was then filtered and the filtrate dried at 100^{o}C and placed in a muffle furnace at 550^{o}C for 4h. Thereafter, the sample was cooled and to it was added 5ml of 5N HCl. The mixture was boiled gently for 10min, cooled and diluted to 100ml with distilled water. Minerals were determined using Atomic Absorption Spectrophotometer (Perkin Elmer, 2380 USA).

The percentage of the total mineral that was soluble in 0.03N HCL was calculated as percentage mineral extractability.

2.6. Data Analysis

All the data generated were analyzed in a one way Analysis of Variance (ANOVA), using the Duncan Multiple Range Test (DMRT) to separate means at $p < 0.05$ level. SPSS package (Statistical Package for Social Sciences) version 21.0, year 2011 was used.

3. Results and Discussion

3.1. Total Carotene Content of the Formulated Complementary Blends

The result showed that the total carotene content of the experimental blends increased as the level of monkey kola increased as presented in Table 2. In the millet-based blends, no carotene was detected in the 100% millet sample (sample A1), indicating that carotene is not found in processed millet. Sample C1 which had no soybean had a higher mean score of 35.43µg/100g carotene than sample B1 (27.6943µg/100g) which had only soybean substitution and no monkey kola. This is an indication that monkey kola was responsible for the increase in total carotene. All other samples increased in their carotene content as substitution increased. Similarly, the maize-based blends increased in their carotene content as substitution level with monkey kola increased. The 100% Maize sample A2, contains some level of carotene. This score decreased in sample B2 as the quantity of maize was reduced to allow for substitution with soybean alone but again increased as monkey kola level increased. Results agreed with the findings of Nwosu *et al*. (2014) who reported an increase in the carotene content of complementary foods when *Moringa oleifera* leaf was blended into maize and soybean. Also, in the millet-based blend, the sample with 25% substitution level of monkey kola was higher than the commercial product while in the maize-based blends, all the samples were better than the commercial product in terms of total carotene but comparing the carotene contents of both blends, the maize-based blends were found to be higher than their corresponding millet-based blends.

Table 2. Total Carotene Content of the Blends on Dry Weight Basis

Sample Codes		Formulation Ratios (%)	Millet-Based Blends (µg/100g)	Maize-Based Blends (µg/100g)
A1	A2	100:00:00	ND	$287.43^{f}\pm25.92$
B1	B2	90:10:00	$27.69^{d}\pm0.55$	$233.61^{e}\pm6.07$
C1	C2	90:00:10	$33.54^{d}\pm1.10$	$346.71^{d}\pm11.58$
D1	D2	80:20:20	$37.44^{d}\pm1.10$	$389.61^{c}\pm0.55$
E1	E2	70:15:15	$64.52^{c}\pm1.41$	$394.68^{c}\pm2.21$
F1	F2	60:20:20	$73.32^{c}\pm2.21$	$434.85^{b}\pm2.76$
G1	G2	50:25:25	$164.58^{a}\pm9.93$	$464.48^{a}\pm19.30$
C3		Control	$133.38^{b}\pm5.52$	$133.38^{g}\pm5.52$
RDI		NA	400.00	400.00

Mean values with the same superscript along a column are not significantly (p<0.05) different, ± standard deviation of duplicate samples. Key: A1 to G1 = Millet-Based Blends, A2 to G2 = Maize-Based Blends, C3 (Control) = Nestle Cerelac Maize, ND = Not Detected, NA = Not applicable**,** RDI = Recommended Daily Intake [*Source: (FAO/WHO, 1998)]

3.2. Total Mineral Composition and Percentage Extractability

Results of the total mineral composition of the complementary blends are shown in Table 3. The calcium contents of the analyzed blends were in the range of 91.01 to 121.59mg/100g, with a percentage extractability of 44.14 to 67.96% while the commercial product had a calcium content of 337.15mg/100g and an extractability of 58.92%. The figures in this work are higher than the findings of Barber *et al.* (2017) who reported a range of 260 to 540mg/kg from their work using maize, soybean and carrot flours to produce complementary foods. The high value of total minerals recorded in the commercial product may be that the commercial product was fortified with calcium compounds like calcium carbonate and also contain skimmed milk. Despite that, samples A1 and F1 were better than the commercial product in terms of calcium absorption. Calcium is a very important mineral; it is required in the formation of strong bone and teeth and its deficiency results in rickets (Kanu *et al.*, 2009). Magnesium is another important mineral and it is requisite with calcium for blood clotting, proper functioning of the lungs and blood pressure regulator (Swaminathan, 2003). It is also a co-factor for enzyme activity (Adeyeye and Agesin, 2007). The analyzed blends have magnesium in the range of 10.44 to 12.29mg/100g. Test samples have better magnesium density than the commercial product. Phosphorous is important in maintaining cellular activities and for bone formation (Berdanier and Zempleni, 2009). Its result ranged from 7.32 to 17.12mg/100g. From the result, the commercial product was not significantly different from sample G2. The iron contents of the analyzed blends were within the range of 9.31 to 26.27mg/100g with an extractability range of 8.19 to 64.81%. Iron helps in the formation of blood cells and also to prevent anaemia. The values for the test samples are lower than the value for the commercial product that served as the control (27.74mg/100g). The reason could be that the commercial product was fortified with ferrous fumarate. However, Dewey and Brown (2003) recommended an iron density of 4.5mg/100g for complementary foods, with a minimum bioavailability of 10%. This means that all the blends but sample F2 met the recommended level. Onabanjo *et al*. (2009) reported lower iron density for complementary food made from carrot and crayfish as well as sorghum and sesame. The higher iron density of the formulated complementary blends in this work could be because of the processing practices employed during the processing of the ingredients. The operations which included soaking/fermentation, boiling, drying and milling are known to reduce the anti-nutrient factors including phytates and polyphenols that tend to inhibit the absorption of iron from plant food origin (Fabbri and Crosby, 2015; Oghbaei *et al*., 2016). The values for zinc content of the analyzed blends were in the range of 1.85 to 6.27mg/100g, all being above the requirement for infants aged 7 - 24 months which is 3mg per day according to USDA (2009). Sample F1 was found to be higher than the value for the commercial products (3.93mg/100g), despite the commercial product being fortified with zinc sulphate. Similar values to those in this work were reported by Onabanjo *et al*. (2009) for four optimized cassava-based complementary foods. Zinc is a vital co-factor for over 70 enzymes; it is implicated in cell mitosis, proper growth and synthesis of protein and body growth. Its deficiency will lead to poor growth, anaemia, liver and spleen enlargement as well as improper development of the skeletal framework. From the findings in this work, it can be concluded that the developed foods can contribute significant amount of minerals to infants.

Table 3. Total mineral content of the blends (mg/100g dry weight basis)

Minerals	A1	A2	F1	F2	G1	G2	C3
Calcium	99.44^{d}±0.02	93.98^{e}±0.01	121.59^{b}±0.09	91.09^{g}±0.01	103.67^{c}±0.02	93.75^{f}±0.02	337.15^{a}±0.01
Magnesium	10.44^{g}±0.01	11.81^{c}±0.01	11.49^{d}±0.01	11.33^{e}±0.05	12.29^{a}±0.06	12.01^{b}±0.01	11.18^{f}±0.01
Phosphorous	11.79^{d}±0.02	12.59^{b}±0.05	7.32^{f}±0.01	9.13^{e}±0.01	12.26^{c}±0.01	17.12^{a}±0.01	17.12^{a}±0.02
Iron	9.31^{g}±0.27	18.05^{d}±0.12	22.77^{c}±0.11	9.99^{f}±0.06	16.39^{e}±0.09	26.27^{b}±0.15	27.74^{a}±0.05
Zinc	2.99^{c}±0.01	2.39^{f}±0.01	6.27^{a}±0.05	1.85^{g}±0.01	2.59^{e}±0.01	2.64^{d}±0.01	3.93^{b}±0.01

Mean values with the same superscript along the same row are not significantly different at P<0.05, ± standard deviations of triplicate samples. Key: A1 = Millet/Soybean/Monkey kola (100:0:0), A2 = (100:0:0), F1 = (60:20:20), F2 = (60:20:20), G1 = (50:25:25), G2 = (50:25:25), C3 = (Control) Nestle Cerelac Maize.

Table 4. Extractable Mineral Content of the Blends (mg/100g Dry Weight Basis)

Minerals	A1	A2	F1	F2	G1	G2	C3
Calcium	67.58^{c}±0.01	54.89^{e}±0.01	81.82^{b}±0.02	40.21^{g}±0.01	60.79^{d}±0.02	43.37^{f}±0.02	198.66^{a}±0.02
Magnesium	10.37^{b}±0.01	10.54^{a}±0.01	10.26^{c}±0.01	9.88^{e}±0.01	10.18^{d}±0.01	10.61^{a}±0.01	9.73^{f}±0.01
Phosphorous	9.57^{b}±0.01	8.84^{c}±0.01	4.20^{g}±0.01	4.96^{f}±0.02	7.82^{d}±0.23	**7.57^{e}**±0.01	10.51^{a}±0.01
Iron	1.90^{f}±0.06	2.98^{e}±0.05	7.83^{d}±0.06	8.18^{d}±0.57	10.62^{b}±0.05	9.07^{c}±0.01	14.28^{a}±0.05
Zinc	1.59^{d}±0.01	1.37^{e}±0.01	4.69^{a}±0.01	1.11^{f}±0.01	1.63^{c}±0.01	1.37^{e}±0.01	1.69^{b}±0.01

Mean values with the same superscript along the same row are not significantly different at P<0.05, ± standard deviations of triplicate samples.

Table 5. Percentage Mineral Extractability Content of the Blends (% Dry Weight Basis)

Minerals	A1	A2	F1	F2	G1	G2	C3
Calcium	67.96[a]±0.01	58.41[e]±0.01	67.33[b]±0.03	44.14[g]±0.01	58.64[d]±0.01	46.26[f]±0.01	58.92[c]±0.01
Magnesium	99.33[a]±0.01	89.27[c]±0.01	89.37[b]±0.01	88.20[e]±0.01	82.56[g]±0.01	88.49[d]±0.02	87.65[f]±0.01
Phosphorous	81.43[a]±0.01	70.24[b]±0.05	57.30[f]±0.01	54.48[g]±0.01	64.92[c]±0.01	59.52[e]±0.01	61.35[d]±0.01
Iron	20.40[d]±0.29	16.43[e]±0.23	34.42[c]±0.07	8.19[f]±0.56	64.81[a]±0.19	34.52[c]±0.20	51.47[b]±0.18
Zinc	53.45[e]±0.06	57.13[d]±0.01	74.71[a]±0.01	60.52[c]±0.06	62.72[b]±0.07	51.62[f]±0.05	42.86[g]±0.01

Mean values with the same superscript along the same row are not significantly different at P<0.05, ± standard deviations of triplicate samples. Key: A1 = Millet/Soybean/Monkey kola (100:0:0), A2 = (100:0:0), F1 = (60:20:20), F2 = (60:20:20), G1 = (50:25:25), G2 = (50:25:25), C3 = (Control) Nestle Cerelac Maize.

4. Conclusion

This research work showed that supplementation of cereal (millet and maize) with 20% soybean and 20% monkey kola flours produced micronutrient dense complementary foods. Similarly, supplementation with 25% soybean and 25% monkey kola flours produced complementary foods that are dense in micronutrients and had good bio-availability percentages. This means that foods formulated from these blends are suitable to improve the micronutrient density of traditional complementary foods and can adequately prevent malnutrition among children of developing countries.

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