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Nutritional Quality and *In vitro* Protein Digestibility of Complementary Foods Formulated from Maize, Cowpea and Orange-Fleshed Sweet Potato Flours: A Preliminary Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Complementary foods were formulated from blends of maize, cowpea and orange fleshed sweet potatoes (OFSP). Five different blends of flour were formulated with the substitution with cowpea flour at 5-30%, and OFSP substitution at 5-20% while 100% maize flour was used as the control. The flour blends were heated and extruded using a cold extruder. The samples were thereafter analyzed for their proximate, mineral, and vitamin compositions, and % In vitro Protein Digestibility. The moisture, ash, fat, crude protein, crude fibre, carbohydrate and energy of the complementary fruits samples varied from 8.11-11.39%, 2.27-3.66%, 2.20-3.10%, 8.87-13.07%, 2.39-4.07%, 68.90-73.43% and 357.08-367.68 Kcal/100g, respectively. All the samples were within the standard RDA for infants and young children, except for fat which was less than 10% recommendation. The

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mineral contents of the complementary food samples ranged from 44.20 – 80.67mg/100g for calcium, 8.86 – 24.50 mg/100g iron, 127.23- 167.72 mg/100 g magnesium, 1.53 -3.17 mg/100g zinc, 31.75 – 63.75 mg/100g phosphorus and 26.86 – 39.98 mg/100g sodium. There were significantly increase ($p < 0.05$) in these minerals as the substitution with cowpea and OFSP flours increased. β -carotene and vitamin C content of the complementary food samples ranged from 10.90 – 31.00 mg/100g and 1.80 – 12.01 mg/100 g, respectively. Increase in substitution with cowpea and OFSP led to an increase in β -carotene values. Vitamin content also increase significantly ($P<0.05$) with increase in proportion of cowpea and OFSP flours. % *in vitro* protein digestibility of the samples varied between 30.29 in MCOA (100% maize complementary food) to 48.77% in sample MCOF (50% maize: 30% cowpea: 20% OFSP). Protein digestibility of the complementary food samples also increased significantly with increase in substitution. Most of the nutrients were highest in the samples containing 20% an 10% OFSP and 30% cowpea and 20% OFSP, making these samples suitable for use as of complementary foods.

Keywords: Complementary foods; cowpea; orange-fleshed sweet potato; maize; proximate; digestibility.

1. INTRODUCTION

“Complementary food is any solid, semi-solid, or soft meal used to fulfill the dietary requirements of an infant when breast milk is no longer sufficient to meet those needs following the exclusive breastfeeding stage” [1]. It is made primarily from cereals such as wheat, maize, and rice, as well as roots and tubers, and legumes. In formulating complementary foods, a single plant product or a variety of plant products can be employed to take advantage of their diverse nutritional qualities and improve the nutritional content of the supplemented food, hence preventing malnutrition issues [2].

Infants are typically given nutrient-fortified cereals, commercial feeding formula, or animal-source meals like milk as their first complementary foods in developed countries; however, these foods are too costly for the vast majority of Nigerian families [3]. As a result, many women have to rely on grain porridges prepared from sorghum, millet, and maize as their only options for feeding their children. Protein and minerals including iron, zinc, iodine, and vitamin A are often lacking in Nigerian newborn supplemental diets [4,5]. These babies are typically given watered-down, high-carbohydrate gruels made from cereals, which are not enough to meet their nutritional demands as they grow.

With an estimated yearly production of roughly 5–6 million tonnes, maize (Zea mays) is the second-most significant grain crop in Nigeria, behind sorghum [6]. 100 grams of edible maize grain contain 10.23 grams of moisture, 8.84 grams of protein, 4.57 grams of fat, 2.33 grams of ash, 2.15 grams of fiber, and 71.88 grams of carbohydrates [7]. It can be used as a raw material to create a variety of foods, including corn meal, breakfast cereals, snacks, and flour [8]. Large amounts of carotenoid pigments found in the yellow maize species are helpful in preventing cancer [9]. Like other cereals, maize is somewhat low in lysine and tryptophan but fair in methionine and cysteine, necessitating supplementation with legume proteins due to the inherent complementing properties of legumes to cereal-based diets.

Cowpea (*Vigna unguiculata*) is a staple food crop grown extensively in West and Central Africa and other tropical and subtropical regions [10]. Its protein content is 25% higher and its protein digestibility is higher than that of other legumes [11]. While it’s nutritional value is enjoyed by people of all socioeconomic backgrounds as a vegetable, sometimes in combination with cereals or grains, and then further transformed into a wide range of manufactured goods [12]. Additionally, they have been used in combination with other cereal grains including maize, millet, and sorghum to create dietary supplements with higher protein content [13].

“Orange-fleshed sweet potato is a biofortified varied variety of sweet potato rich in provitamin A carotenoids which can be used to combat malnutrition and vitamin A deficiency in small and marginal farming community” [14]. “One medium sized of orange-fleshed sweet potato can provide about twice the β-carotene needed for the daily requirement of vitamin A” [15]. “Orange-fleshed sweet potato flour has also been used in production and enrichment of baked products such as cakes, bread, and buns” [16]. Thus, the production of a nutrient rich and acceptable complementary food with a blend of cowpea and orange-fleshed sweet potato can be a better approach to meet the nutrient and energy needs of infants. Therefore, the aim of study was to evaluate the nutrient quality and invitro protein digestibility of complementary foods developed from Maize, Cowpea and Orange-Fleshed Sweet Potato flours.

2. MATERIALS AND METHODS

2.1 Materials

Orange-fleshed sweet potato (*Ipomea batatas*), yellow variety of maize (*Zea mays*) and cowpea (*Vigna unguiculata* L. Walp) were purchased from Mile 3 market in Port-Harcourt Nigeria. Chemicals used in this study were of analytical reagent grade.

2.2 Production of Maize Flour

Maize flour was produced according to the method described by [17], as shown in Fig. 1. Five kilograms (5 kg) of maize grains was sorted and washed with clean water. Thereafter the washed maize grains were dried in a hot air oven at 60°C for 24 hrs. The dried maize grains were milled, sieved with 0.2 mm sieve, and stored in a well-labeled transparent plastic container until further use.

2.3 Production of Orange-Fleshed Sweet Potatoes Flour (OFSP)

Orange-fleshed sweet potatoes flour was prepared according to the method of [18], as shown in Fig. 2. The Orange Fleshed Sweet Potatoes (1.5 kg) was washed with clean water to remove dirt and other foreign materials. They were then peeled using a knife, cut into cubes of about 1.5 cm, washed using distilled water and oven dried at 50°C for 24 hrs. Samples were left to cool at room temperature and then dried and milled into powder and sieved with (0.4 mm) sieve aperture into flour. The flour produced was packaged in an airtight plastic container and labeled until needed for further use.

2.4 Production of Cowpea Flour

Cowpea flour was prepared according to the method described by Keyata et al. [19], as shown in Fig. 3. Ten kilograms (10 kg) of cowpea seeds was weighed after cleaning and sorting. Cleaned seeds were washed in distilled water (1:2 w/v). Thereafter, the seed coats were removed and manually rinsed in clean water until they were free from impurities. They were oven dried at 70 °C for 24 hrs, left to cool at room temperature and milled using a hammer mill. The flour was kept in a dry, air-tight plastic container for further use.

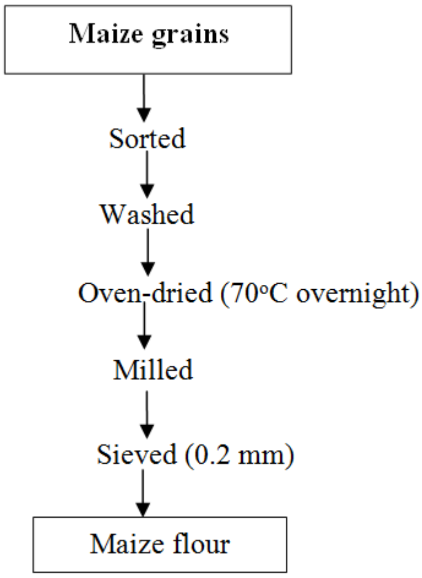

Fig. 1. Production of maize flour
Source: [17]

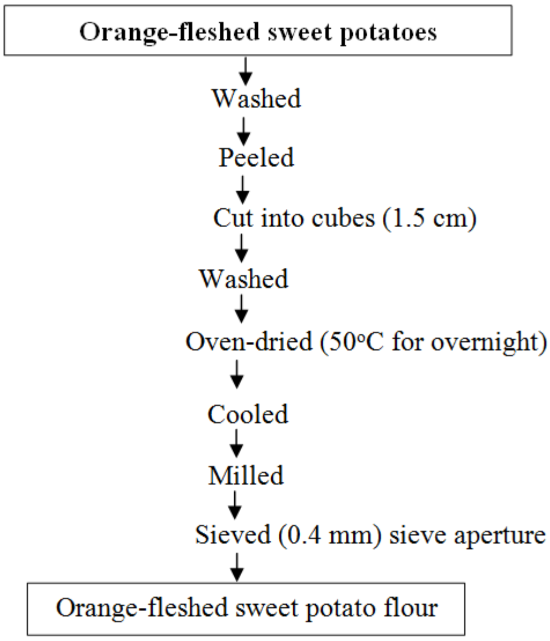

Fig. 2. Production of orange fleshed sweet potatoes flour
Source: [18]

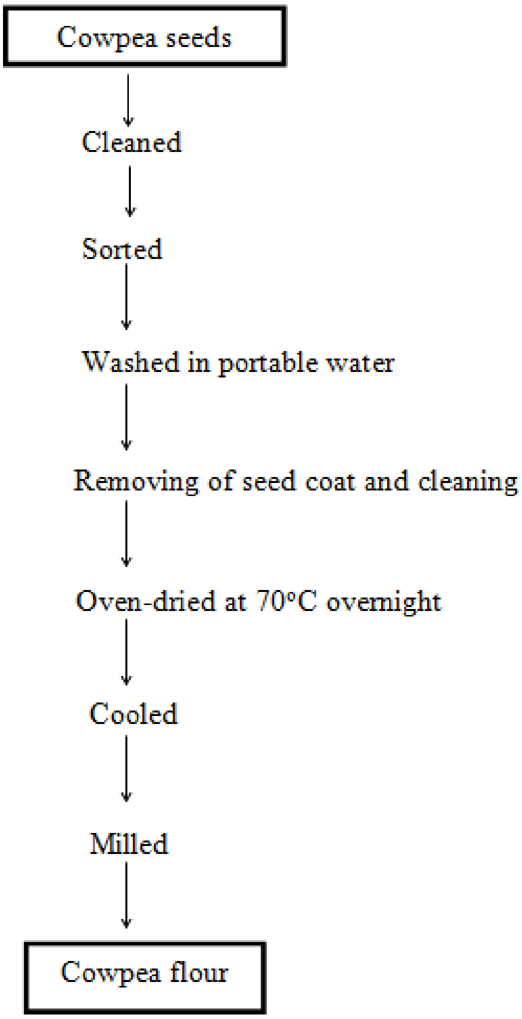

Fig. 3. Production of cowpea flour
Source: [19]

2.5 Formulation of the Flour Blends and Experimental Design

The experimental design as planned according to a full factor factorial arrangement in a completely randomized design (CRD) with three types of flour: maize flour (MF), cowpea flour (CF) and orange-fleshed sweet potato flour (OFSPF). Six formulations were made to produce six coded samples in the following ratios: MCOA= 100: 0: 0; MCOB=90:5:5; MCOC= 80:15:5; MCOD= 70:20:10; MCOE= 60:25:15; MCOF= 50:30:20. The ratios represent the inclusion of the flours in this order, MF: CF: OFSPF. The experimental design is shown in Table 1.

2.6 Preparation of Complementary Foods

The flour blends were thoroughly mixed with 5 L of water and heated on a stove for 1 hr, and then allowed to cool at room temperature. They were then fed into a laboratory size cold extruder, dried in an oven at 50°C for 12 h. The samples were then crushed in a mortar and transferred into an air tight container for further use.

2.7 Proximate Analysis

"The [20] method which included moisture, ash, fat, crude protein and crude fibre was used to determine the proximate composition of the complementary foods. Ash was measured using a muffle furnace (Sanyo Gallenkamp, Weiss Technik, West Midlands, UK) heated to 550°C for 6 hours. Fat was calculated using soxhlet method and crude protein using kjeldhal method and nitrogen multiplied by a conversion factor of 6.25. Crude fibre was measured gravimetrically and carbohydrate obtained by subtraction (moisture, ash, fat, crude protein and crude fibre) from hundred percent".

2.8 Determination of Energy Content

The energy content (E) of the complementary food was calculated using Atwater factor as described by [21]

$$\text{Energy (Kcal/100g)} = (4 \times \text{Protein}) + (9 \times \text{Fat}) + (4 \times \text{Carbohydrate}) \quad \text{Eqn 1.}$$

2.9 Mineral Analysis

Dry ashing, as described in [20], was used for the mineral analysis. The sample, which weighed 2 g, was ashed in a crucible at 550°C for 2 hours. First, the ashed sample was dissolved by adding 5 ml of concentrated hydrochloric acid (HCL), then 20 mL of deionized water, and finally heated to stop the reaction. After letting the solution cool, it was filtered through Whatmat No. 1 filter paper and brought to a final volume of 50 ml. After collecting and cleaning the samples, an atomic absorption spectrophotometer (AAS) was used to determine the total mineral content (calcium, magnesium, zinc, and iron) (Buck Scientific - 210VGP, USA). The UV-VIS spectrophotometer was used to determine the concentration of calcium, iron, magnesium and zinc at 700 nm while phosphorus was determined using molybdenum blue method (CELIL model CE2021 U.K). Sodium was analyzed by flame spectrophotometer (Jenway model PFP7/C). The amount of minerals in the samples was calculated, and the percentage of minerals in each 100g sample as given in milligrams.

Metal (g/100g) = $\frac{Concentration\ (ppm) \times Solution\ volume}{104 \times Sample\ weight}$ Eqn 2.

Metal(mg/100g) = Metal(g/100g)×1000 Eqn 3.

2.10 Determination of Total Carotenoid Content

Total carotenoid content of the complementary foods were determined by the method described by [22]. This method involved filtering of 10g of the sample into a conical flask and addition of 50ml of 95% ethanol. The flask and the content are placed in a shaking water bath set to 70-80°C for 20min, allowed to cool and the supernatant decanted into a measuring cylinder and the initial volume V_1 is recorded. The ethanol concentration of the mixture is brought to 85% by adding 15ml of distilled water, allowed to cool in ice or at room temperature standing. 25ml of petroleum ether is added to the cooled ethanol extract, transferred the cooled separately funnel and shaken to obtain a homogenous mixture. It is then allowed to separate out into two layers, the bottom layer is collected in a beaker back into the separating funnel and re-extracted with 10ml petroleum ether for about 5 times or continuously until the extract becomes fairly yellow. The petroleum extract mixture is then collected in a beaker back into the emptied separating funnel and re-extracted with 50ml of 80% ethanol, the final volume V_2 is recorded and the extract stored for spectrophotometric determination in the dark. The absorbance of the extract is then read using a UV visible spectrophotometer at 436nm. Petroleum ether is used as blank.

Beta carotene = $\frac{\text{Abs x Vt}}{\text{Ec x 1 x W}}$ Eqn 4

Abs = Absorbance
Vt =Total volume of petroleum ether extract from which aliquot was taken and analysed on spectrophotometer.
Ec = Extinction coefficient of β-carotene (12.50mg/l)
1 = Is the cuvette thickness/path length
W = Weight of the sample

2.11 Determination of Vitamin C Content

Vitamin C concentrations were measured using the technique outlined by [20]. The material was weighed out at 5.0 grams and placed in a clean beaker with 112.5 milliliters of distilled water and 12.5 milliliters of oxalic acid. At room temperature, this was shaken for 30 minutes before being filtered and topped off to 50 ml. The sample was pipetted into a 100 ml volumetric flask, and titrated with the indophenol solution until a very faint pink color lasted for 15 seconds. The concentration was expressed as mg ascorbic acid equivalent to Vml of the dye solution i.e. 10ml ascorbic acid solution = 0.002g ascorbic acid. If 0.002g ascorbic acid required 1ml dye solution to neutralize it then 1ml dye solution =,

Vit C = $\frac{V \times T}{W}$×100g Eqn 5.

V= ml dye used for titrating of aliquot of sample
T= Ascorbic equivalent or dye solution expressed as ml/ml
W= gm of sample in aliquot titrated

2.12 *In Vitro*-Protein Digestibility (IVPD)

IVPD was carried out according to the method described by Minjula and John [23] as reported by F with minor modification. 2.5 g of sample containing sixteen mg (16 mg) was taken in triplicate and digested with 1mg pepsin (Cat no. P6887, Sigma Chemicals Ltd USA) in 15 ml of 0.1N NCL at 37°C for 2h. The reaction was stopped by the addition of 15ml 10% trichloroacetic acid (TCA). The mixture was then filtered quantitatively, through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro – Kjeldah method (AOAC, 2000). Digestibility was estimated by using the following equation.

IVPD(%) = $\frac{N\ in\ supernatant - Enzyme\ N}{N\ in\ sample} \times 100$ Eqn 6.

2.13 Statistical Analysis

Results were expressed as mean values and standard deviation of triplicate was determined. Data were analyzed using a one-way analyses of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20.0 software 2011 to test the level of significance (p<0.05). Turkey HSD Test was used to separate the means where significant differences exist as described by Wahua [24].

Table 1. Formulation of the flour blends (g)

Sample	Maize flour	Cowpea flour	Orange-fleshed sweet potato flour
MCOA	100	-	-
MCOB	90	5	5
MCOC	80	15	5
MCOD	70	20	10
MCOE	60	25	15
MCOF	50	30	20

Keys: MCOA= 100% Maize; MCOB= 90% maize: 5% cowpea: 5% OFSP; MCOC= 80% maize: 15% cowpea: 5% OFSP; MCOD= 70% maize: 20% cowpea: 10% OFSP; MCOE= 60% maize: 25% cowpea: 15% OFSP; MCOF= 50% maize: 30% cowpea: 20% OFSP

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Complementary Foods Developed from Maize, Cowpea and Orange-Fleshed Sweet Potato Flour Blends

Table 2 showed the proximate composition of complementary foods developed from maize, cowpea and orange-fleshed sweet potato flour blends. Moisture content of the samples were as follows: 9.96%, 11.39%, 9.72%, 9.16%, 8.64%, and 8.11%, for samples MCOA, MCOB, MCOC, MCOD, MCOE, and MCOF, respectively. Moisture content of sample MCOB was significantly (p<0.05) different from other samples except sample MCOA and MCOC. Increase in the proportion of cowpea and sweet potato resulted in a significant (p<0.05) decrease in the moisture content of the samples. The values obtained in this study are similar to the values of 7.56-8.35% reported by [25] for complementary foods from malted millet and protein isolate of pumpkin. Therefore, the moisture level of the complementary foods studied (with the exception of sample MCOB) was below the 10% threshold recommended by the United Nations' protein Advisory Committee to maximize the shelf life of such items. The low residual moisture content (10%) of the complementary foods is favorable since it reduces microbial proliferation and increases storage life.

“Ash content of the samples ranged from 2.27-3.66% with MCOA recording the lowest value (2.27%) while MCOF had the highest (3.66%). There was a significant (p<0.05) increase in the ash content of the samples as substitution with cowpea and orange fleshed sweet potato flour increased. Ash content of MCOF was significantly (p<0.05) different from MCOA, MOCB, and MCOC but not from MCOE. The increase in ash content observed in the samples supplemented with cowpea and orange fleshed sweet potato flours may be attributed to high mineral content of cowpea” [11]. “The result obtained is higher than the range of 0.56-2.00% for complementary foods formulated from different blends of maize, soybean and carrot flour” [2]. “Ash content of a food material could be used as an index of mineral constituents of a food material” [26]. “The ash values obtained from this study (2.27-3.66%) meets the values of <5% recommended by codex for ash in formulated complementary foods for infants and young children” [27].

“Fat content of the samples decreased significantly (p<0.05) as the proportions of cowpea and orange fleshed sweet potato flours increased. MCOF recorded the highest fat content of 3.10%, while MCOA had the lowest the 2.20%. Fat content of MCOA was significantly (p<0.05) different from other samples except from MCOB and MCOC. The decrease in fat content from this study is expected as sweet potato is not good source of fat. The fat content in this study is also lower than recommended 10% for complementary foods” [28]. Although fat levels obtained is low, low fat content in the diets may be necessary to prolong its shelf life also by avoiding lipid peroxidation [29].

The highest crude protein value of 13.07% was recorded in MCOF, while MCOA had the lowest value of 8.87%. There was a significant (p<0.05) difference in the protein contents of MCOC, MCOD, MCOE and MCOF while MCOA and MCOB exhibited similar protein content with no significant (p>0.05) difference observed. Increase in the levels of cowpea and orange fleshed sweet potato substitution led to a significant (p<0.05) increase in the protein content of the sample. The observed increase is in agreement with the study of [11] who reported that cowpea has high protein digestibility and is a good source of protein. However, recommended protein content for complementary foods is 15% [28]. In this study, all the formulated samples had protein contents below the recommended value. The implication is that the complementary foods would have to be enriched with protein rich foods such as sea foods and other legumes to enhance the protein as protein content is important for growth of the infant [30].

"Crude fibre content of the samples ranged from 2.39-4.07%, with MCOA having the lowest while MCOF had the highest value. Crude fibre content of MCOF was significantly ($p<0.05$) different from all other samples except from MCOE. Crude fibre content of MCOC, MCOD and MCOE were also significantly ($p<0.05$) different. The incorporation of cowpea and orange fleshed sweet potato flour led to a significant ($p<0.05$) increase in the crude fibre content of the samples. The increase could be attributed to the high crude fibre of cowpea (5.75%)" as reported by [10]. The crude fibre content of all the samples were within the recommended values of <5% fibre [28]. Due to their low fiber content, these samples might be consumed in large quantities during complementary feeding, giving infants a better chance to achieve their daily energy and other vital nutrient needs.

Carbohydrate content of the samples ranged from 68.90-73.43% with the lowest value (68.90%) obtained in MCOF while sample MCOA had the highest (73.43%). There was no significant ($p>0.05$) difference in the carbohydrate contents of MCOA, MCOC and MCOF. Increase in the levels of cowpea and orange fleshed sweet potato substitution led to a significant ($p<0.05$) reduction in the carbohydrate of the samples. The decrease in the carbohydrate content could be principally due to the low carbohydrate content of cowpea over maize. Carbohydrate values obtained from this study is higher than the values of 32.56-37.33% obtained by [31] for complementary food formulated from sorghum, sesame, carrot and crayfish. The relatively high carbohydrate from this study is an indication that the formulated complementary foods would provide the infants with adequate amount of energy. The carbohydrate values of the studies complementary foods samples were within the WHO/FAO [28] (> 65%) standard for complementary foods for infants and young children.

Energy content was highest in MCOA with a value of 357.08 Kcal/100g while MCOF had the lowest value (347.68 Kcal). Energy content of MCOA was significantly ($p<0.05$) different from MOCE. No significant ($p>0.05$) differences existed in the energy contents of MCOB, MCOC, MCOD and MCOE. Increase in the proportion of cowpea and orange fleshed sweet potato flour also led to a significant ($p<0.05$) reduction in the energy content of the samples; however MCOA showed an increase in the energy content, but there was no significant difference ($p>0.05$) between MCOB, MCOC, MCOD and MCOE. The observed differences in the energy values of the formulations could be attributed to variation in the protein, fat and carbohydrate contents of the samples. The decrease could also be due to the low fat and carbohydrate contents of the blends as both constitute major source of energy. The energy values obtained in this study were in agreement with [32]. 344 – 370 kcal/100g Standard for Infant and Young Children.

Table 2. Proximate composition of complementary food samples

Samples	Moisture (%)	Ash (%)	Fat (%)	Crude protein (%)	Crude fibre (%)	CHO (%)	Energy (Kcal/100g)
MCOA	$9.96^{ab}\pm0.12$	$2.27^{c}\pm0.09$	$3.10^{a}\pm0.01$	$8.87^{e}\pm0.14$	$2.39^{d}\pm0.04$	$73.43^{a}\pm0.12$	$357.08^{a}\pm0.91$
MCOB	$11.39^{a}\pm1.20$	$2.39^{c}\pm0.03$	$2.95^{ab}\pm0.08$	$9.11^{e}\pm0.06$	$2.63^{cd}\pm0.06$	$71.54^{ab}\pm1.19$	$349.13^{ab}\pm2.24$
MCOC	$9.72^{ab}\pm0.13$	$2.58^{bc}\pm0.05$	$2.71^{abc}\pm0.25$	$9.81^{d}\pm0.11$	$2.90^{c}\pm0.17$	$72.30^{b}\pm0.21$	$352.75^{ab}\pm2.62$
MCOD	$9.16^{b}\pm0.33$	$2.74^{bc}\pm0.10$	$2.61^{bcd}\pm0.02$	$10.71^{c}\pm0.25$	$3.38^{b}\pm0.10$	$71.41^{ab}\pm0.36$	$351.90^{ab}\pm0.62$
MCOE	$8.64^{b}\pm0.14$	$3.10^{ab}\pm0.01$	$2.27^{cd}\pm0.07$	$11.97^{b}\pm0.05$	$3.83^{a}\pm0.09$	$70.20^{bc}\pm0.06$	$349.09^{ab}\pm0.21$
MCOF	$8.11^{b}\pm0.32$	$3.66^{a}\pm0.33$	$2.20^{d}\pm0.04$	$13.07^{a}\pm0.06$	$4.07^{a}\pm0.08$	$68.90^{c}\pm0.10$	$347.68^{b}\pm0.55$
LSD	1.63	0.44	0.35	0.40	0.31	1.60	6.44

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at ($p<0.05$)

KEYS: MCOA= 100% Maize; MCOB= 90% maize: 5% cowpea: 5% OFSP; MCOC= 80% maize: 15% cowpea: 5% OFSP; MCOD= 70% maize: 20% cowpea: 10% OFSP; MCOE= 60% maize: 25% cowpea: 15% OFSP; MOCF= 50% maize: 30% cowpea: 20% OFSP; LSD= Least Significant Difference

3.2 Vitamin Composition of Complementary Foods

Table 3 shows the vitamin composition of complementary food samples**.** For β-carotene content of the samples, MCOF had the highest value (31.00 mg/100g) while MCOA had the lowest (10.90 mg/100g). There was no significant ($p>0.05$) difference in the β-carotene content of MCOD, MCOE and MCOF. Similar trend was also observed for MCOB and MCOC. A significant ($p<0.05$) increase in the β-carotene content was observed as the proportion of OFSP and cowpea flour increased. This increase is attributed to the substitution with orange-fleshed sweet potato as [33] reported that β-carotene is high in the orange fleshed sweet potato. [34] also reported that orange fleshed sweet potato contains 24.2-73.9 mg/100g of β-carotene on dry basis. Their high beta-carotene content helps prevent or treat vitamin A deficiency. Because of the higher levels of beta-carotene found in the complementary foods, infants in low-resource agricultural communities will have a better chance of avoiding malnutrition and vitamin A deficiency. Children should get between 30 and 150 mg of beta-carotene each day, according to the RDA [35]. In this study, only MCOF containing 50% maize: 30% cowpea: 20% OFSP met the recommended dietary allowance. Although, these RDA can also be met in other samples if a child consumes at least 300 g of the sample each day.

“Vitamin C content of the samples was highest in MCOF with a value of 12.01 mg/100g, and lowest in MCOA 1.80 mg/100g). Vitamin C content of MCOF was significantly ($p<0.05$) different from all other samples while MCOA and MCOB did not differ significantly ($p>0.05$) from each other. It was observed that Vitamin C content of samples increased significantly ($p<0.05$) as the substitution with cowpea and OFSP flour increased. The increase is due to the substitution with OFSP as orange-fleshed sweet potato is an excellent source of vitamins” [36]. “Vitamin C content of the samples was comparable with the values of 2.34-50.41 mg/100g obtained by [37] for complementary foods produced from maize, soybean and banana”. “The recommended dietary allowance for RDA for vitamin C in children aged 0-3 years is between 15-50 mg/day” [35]. “The low vitamin C content in the samples could have been due to the extrusion process. During extrusion, factors like barrel temperature, screw rpm, die diameter and throughput affect the retention of vitamins in foods and the most sensitive to the extrusion process are vitamin C” [38]. None of the samples fell within the recommended standard, thought it can be achieved if a child consumes at least 200g of MCOD, MCOE and MCOF.

Table 3. Vitamin composition of complementary food samples (mg/100g)

Samples	β-carotene	Vitamin C
MCOA	$10.90^{c}±0.28$	$1.80^{d}±0.13$
MCOB	$21.10^{b}±0.71$	$2.07^{d}±0.04$
MCOC	$21.90^{b}±2.12$	$6.00^{c}±0.07$
MCOD	$26.70^{a}±1.13$	$8.95^{b}±0.11$
MCOE	$28.95^{a}±0.07$	$10.21^{b}±0.39$
MCOF	$31.00^{a}±0.85$	$12.01^{a}±0.83$
LSD	3.34	1.16

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at (p <0.05); KEYS: MCOA= 100% Maize; MCOB= 90% maize: 5% cowpea: 5% OFSP; MCOC= 80% maize: 15% cowpea: 5% OFSP; MCOD= 70% maize: 20% cowpea: 10% OFSP; MCOE= 60% maize: 25% cowpea: 15% OFSP; MCOF= 50% maize: 30% cowpea: 20% OFSP; LSD= Least Significant Difference

3.3 Total Minerals of Complementary Foods

Table 4 shows the total minerals (mg/100g) of complementary food samples. Calcium content was found to be highest in MCOF with a value of 80.87 mg/100g and lowest in MCOA with a value of 44.20 mg/100g. There was significant ($p<0.05$) difference in the calcium content of the complementary foods. There was a significant ($p<0.05$) increase in the calcium content of the samples as the proportion of cowpea and OFSP increased. The increase in calcium contented could be attributed to increase in the addition of cowpea flour and OFSP in the blends. Calcium content of the formulated complementary foods from this study was lower than [39] recommended value of calcium (341.2 mg/100g). It is however within the values of 12.68-84.86 mg/100g obtained by [40] for complementary foods formulated from sorghum, African yam bean and crayfish. In infants and young children, calcium plays an important role in bone and tooth development, muscle and nerve function, blood coagulation, and immune system health [41].

Iron content of the samples ranged from 8.86 mg/100g in MCOA to 24.50 mg/100g in MCOF. There was significant ($p<0.05$) difference in the iron content of the complementary foods. Similarly, there was a significant ($p<0.05$) increase in the iron content of the complementary foods as the proportion of cowpea flour and OFSP increased. The results are slightly higher than the iron content of complementary foods produced from sorghum, African yam bean and crayfish flours (2.68-7.9 mg/100g) as reported by [40]. The increase in the iron content of the complementary foods on substitution with cowpea and OFSP flour is good for infants and children since regular consumption of food rich in iron has the potential to prevent anaemia in infants and young children. The value of iron from this study is also within the nutritional requirement for iron by children which is 8-18 mg/day [28].

MCOA had the highest magnesium content (167.72 mg/100g) while MCOF had the lowest value (127.23 mg/100g). There was significant ($p<0.05$) difference in the magnesium content of the complementary foods. These values were higher when compared to the values of 12.13-96.50 mg/100g reported by [42] for complementary foods produced from popcorn, soybean cake and wonderful kola flour blends. Magnesium helps in the proper functioning of the muscles. It also serves as an activator in many enzyme systems [43]. The FAO/WHO daily magnesium requirement for infants is 0.04 mg/100g [28]. This is met in all the food samples.

Zinc content was highest in MCOF with a value of 3.17 mg/100g while MCOA had the lowest value for zinc (1.53 mg/100g). There was significant ($p<0.05$) difference in the zinc content of the complementary foods. Similarly, the high zinc content in MCOF is attributed to the substitution with OFSP and cowpea flour. In addition to its key involvement in cell division, protein synthesis, and growth, zinc is a co-factor for more than 70 enzymes. Failure to thrive, anemia, enlarged organs (including the liver and spleen), and stunted growth and development are all the results of lack of zinc in complementary foods [43]. The values of zinc obtained from this study were lower than the recommended values of 8-14 mg/day for children [28], although these values could be met if the infant consumes at least 300g of MCOC, MCOD, MCOE and MCOF. However, it was within the range (0.12-8.00 mg/100g) obtained [42]) for complementary foods produced from popcorn, soybean cake and wonderful kola flour.

Phosphorus content of the samples ranged from 31.75 mg/100g in sample MCOA to 68.75 mg/100g in MCOF. There was significant ($p<0.05$) difference in the phosphorus content of the complementary foods. Increase in phosphorus values of the samples could be due to the increase in the proportion of OFSP and cowpea flour. Phosphorus content of the formulated complementary foods was lower than [37] recommended value (281.20 mg/100g). However, these values were higher when compared to the values (5.51 - 8.02 mg/100g) obtained by [44] on complementary foods supplemented with black bean and crayfish flours. It is also higher than the phosphorus content (4.17 - 27.93 mg/100g) of complementary foods formulated from yellow maize, soybean, millet and carrot flours [45] Phosphorus is an important constituent of every living cell. It is also essential for the formation of the bone.

Sodium content was highest in MCOF with a value of 39.98 mg/100g and then lowest (27.84 mg/100g) in MCOE. There was significant ($p<0.05$) difference in the sodium content of the complementary foods. There was a significant ($p<0.05$) increase in the sodium content of the flour blends as the proportion of OFSP and cowpea flour in the blends increased. The sodium content of the food samples did not meet with WHO [32] sodium requirement of infant's (112.01 mg/day). The range of values from this study is also lower than those (45.87 - 80.44mg/100g) reported by [25] for complementary foods produced from millet and enriched with protein isolate of fluted pumpkin seed. Sodium is an essential nutrient necessary for the maintenance of plasma volume, acid-base balance and normal cell functions [46].

Table 4. Total minerals (mg/100g) of the complementary foods

Samples	Ca	Fe	Mg	Zn	P	Na
MCOA	$44.20^{f}\pm0.01$	$8.86^{f}\pm0.01$	$167.72^{a}\pm0.01$	$1.53^{f}\pm0.00$	$31.75^{f}\pm0.01$	$26.86^{f}\pm0.01$
MCOB	$48.69^{e}\pm0.03$	$9.80^{e}\pm0.01$	$161.91^{b}\pm0.01$	$1.67^{e}\pm0.01$	$33.75^{e}\pm0.01$	$27.84^{e}\pm0.01$
MCOC	$60.60^{d}\pm0.01$	$10.40^{d}\pm0.02$	$151.47^{c}\pm0.01$	$2.17^{d}\pm0.01$	$42.55^{d}\pm0.01$	$30.40^{d}\pm0.01$
MCOD	$66.58^{c}\pm0.01$	$12.40^{c}\pm0.01$	$149.47^{d}\pm0.02$	$2.68^{c}\pm0.01$	$51.25^{c}\pm0.01$	$33.14^{c}\pm0.02$
MCOE	$79.81^{b}\pm0.01$	$19.31^{b}\pm0.01$	$139.72^{e}\pm0.01$	$3.11^{b}\pm0.01$	$56.25^{b}\pm0.01$	$37.35^{b}\pm0.01$
MCOF	$80.87^{a}\pm0.01$	$24.50^{a}\pm0.01$	$127.23^{f}\pm0.01$	$3.17^{a}\pm0.01$	$68.74^{a}\pm0.01$	$39.98^{a}\pm0.01$

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at ($p<0.05$); Keys: A= 100% Maize; B= 90% maize: 5% cowpea: 5% OFSP; C= 80% maize: 15% cowpea: 5% OFSP; D= 70% maize: 20% cowpea: 10% OFSP; E= 60% maize: 25% cowpea: 15% OFSP; F= 50% maize: 30% cowpea: 20% OFSP

3.4 *In vitro* Protein Digestibility

Table 5 shows the in vitro protein digestibility of the complementary foods. % invitro protein digestibility of the complementary food ranged from 30.29% in MCOA and 48.77% in MCOF. IVPD of MCOF was significantly ($p<0.05$) different from other samples while MCOA and MCOB did not differ significantly ($p>0.05$) from each other. There was significant increase ($P < 0.05$) in the IVPD of the samples with sample MCOF (48.77%) having higher IVPD than all the samples. The high protein digestibility in samples substituted with higher proportion of Cowpea flours (20 -30%) could be attributed to the high protein composition of cowpea than maize. The result obtained in this study is lower than that obtained by [47] for protein digestibility of complementary food (72.51 – 82.17%) formulated from guinea corn, maize, sorghum, millet, groundnut and soybean. The result is also comparable with [48] who reported an increase in IVPD in complementary food formulated from rice with different proportions of sprouted green gram. It's also in agreement with those obtained by [49] for green gram and [50] for infant cereals. Increase in protein digestibility of this study might be due to either reduction of anti-nutritional factors and/ or denaturation of proteins making them more available to proteolytic enzyme activity [51].

The nutritional quality of any protein relates to its amino acid composition, digestibility and ability to supply the essential amino acids in the amount required by the species consuming the protein [52]. Protein is needed as building blocks for the body, necessary for growth and for the repair of damaged tissues [53].

Table 5. Protein digestibility (%) of the complementary foods

Samples	IVPD (%)
MOCA	$30.29^{d} \pm 0.02$
MCOB	$31.31^{d} \pm 0.69$
MCOC	$39.51^{c} \pm 0.01$
MCOD	$43.29^{b} \pm 0.01$
MCOE	$43.79^{b} \pm 0.02$
MCOF	$48.77^{a} \pm 0.76$
LSD	1.15

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at (p <0.05); Keys: MCOA= 100% Maize; MCOB= 90% maize: 5% cowpea: 5% OFSP; MCOC= 80% maize: 15% cowpea: 5% OFSP; MCOD= 70% maize: 20% cowpea: 10% OFSP; MCOE= 60% maize: 25% cowpea: 15% OFSP; MCOF= 50% maize: 30% cowpea: 20% OFSP; LSD = Least Significant Difference

4. CONCLUSION

Complementary foods were successfully formulated using Maize, Cowpea and OFSP. The complementary foods showed nutritional values higher than the control. The proximate composition of all the samples were within the recommended values except for fats which was lower than the recommended value. The mineral values of all the complementary foods were less than the recommended values except for iron (8-18mg/day) and magnesium (0.04mg/day) which were within the recommended values. The vitamins contents of complementary food were also less than the recommended value except for β-carotene in sample with 30% cowpea and 20% OFSP of the complementary food. Although these values can be reached if an infant consumes more than 300 g daily which an infant is likely to consume. %IVPD increased as a result of increase in cowpea substitution, since cowpea is rich in protein. There was significant increase in the nutritional composition of the complementary food as the substitution of OFSP, and Cowpea flours were increased. The concentration of these minerals were found to be highest in samples, substituted with 25% cowpea and 15% OFSP, 30% cowpea and 20%, indicating that these samples are suitable for use as complementary foods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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