

Original Research Article

Comparative Analysis of Nutrient and Anti-Nutrient Profiles in Complementary Foods formulated from Yellow Maize, Sorghum, Millet, and Soybeans fortified with Walnuts, Pumpkin Seeds, and Date Palm Fruits

ABSTRACT

The study evaluates and compares the nutrient and anti-nutrient profiles of complementary foods formulated from yellow maize, sorghum, millet, and soybeans, fortified with walnuts, pumpkin seeds, and date palm fruits. The raw materials underwent fermentation, sprouting, boiling, blanching, and toasting before formulation. Nutrisurvey2007 software was used to optimize the food formulations, and a cost analysis was performed. Cerelac, a commercial complementary food, served as the control. The three complementary food formulations (CFF1, CFF2, and CFF3) were analyzed for proximate, mineral, vitamin, and anti-nutritional content using standard analytical methods and compared against the World Health Organization (WHO) and Codex Alimentarius standards. The results revealed significant ($p < 0.05$) differences in nutrient composition between Cerelac and CFF. Protein content in CFF ranged from 10.30% (CFF1) to 11.80% (CFF3), closely matching Cerelac's 11.90% and aligning with WHO's recommendation of 10–15% for optimal growth. Both Cerelac and CFF provide essential minerals and CFF formulations, particularly CFF2 and CFF3, demonstrate superior content in key minerals like calcium, magnesium, and iron, contributing to enhanced dietary adequacy. For example, iron content was significantly ($p < 0.05$) higher in CFF2 (11.70 mg/100 g) compared to Cerelac (6.62 mg/100 g), meeting WHO's daily requirement of 3.9 to 11.6 mg/100g. Vitamin B12 levels in CFF2 (4.69 $\mu\text{g}/100\text{ g}$) and CFF3 (4.43 $\mu\text{g}/100\text{ g}$) significantly ($p < 0.05$) exceeded those in Cerelac (1.05 $\mu\text{g}/100\text{ g}$), surpassing the Recommended Nutrient Intake (RNI) of 0.9 $\mu\text{g}/\text{day}$. Phytate levels in Cerelac (4.78 mg/g) and CFF (3.45–4.36 mg/g) in CFF2 and CFF3 remained below the Codex Alimentarius threshold of 5 mg/g. These findings demonstrate that the complementary foods formulated from locally available raw materials are nutrient-dense, affordable, and meet key international nutritional standards, offering a viable alternative for addressing malnutrition in resource-limited settings.

Keywords: Complementary foods; local raw materials; fortification; nutrients; anti-nutrients.

1. INTRODUCTION

Malnutrition remains a significant health issue globally, with many children in resource-limited regions failing to meet their nutritional needs due to limited access to commercial complementary foods. According to the Food and Agriculture

Organization (FAO), more than 149 million children under five are stunted, primarily due to inadequate nutrition (FAO, 2020). Complementary feeding, introduced around six months of age alongside breastfeeding, must provide essential nutrients to bridge dietary gaps left by breast milk alone (WHO, 2020). In many developing countries including Nigeria, commercial complementary foods are too costly for low-income families. This has increased interest in formulating affordable, nutrient-rich complementary foods using locally available raw materials. It offers a sustainable, culturally appropriate solution for infant nutrition, reduces imports, and strengthens local economies. Many traditional crops and foods have nutritional qualities that benefit child nutrition. These crops, widely grown and familiar to local communities, are rich in macronutrients, vitamins, and minerals. Cereals, legumes, nuts, seeds, and fruits offer a wide range of nutrients that can be combined to meet the dietary needs of infants (Albuquerque *et al.*, 2020). Cereals like yellow maize, sorghum, and millet, provide energy but lack essential amino acids, vitamins, and minerals (Obinna-Echem *et al.*, 2018). Soybeans are rich in plant-based protein and essential fatty acids but require processing to reduce anti-nutritional factors such as phytates and trypsin inhibitors, which hinder nutrient absorption (Adebiyi *et al.*, 2017). Fortifying these traditional staples with nutrient-dense seeds, nuts, and fruits can address the dietary inadequacies inherent in cereal-based complementary foods. Walnuts and pumpkin seeds are rich in essential fatty acids, protein, and minerals such as zinc and magnesium, which are necessary for growth and cognitive development (Chauhan and Chauhan, 2020). Date palm fruits provide natural sugars, dietary fiber, and vitamins A and C, which enhance immune function (Alfarsi *et al.*, 2023). These local raw materials are often abundant and inexpensive but require appropriate processing and formulation to meet dietary requirements. Processing techniques such as fermentation, sprouting, and toasting are necessary for optimizing the bioavailability of nutrients and reducing anti-nutritional factors in raw materials (Samtiya *et al.*, 2020). Fermentation increases the levels of B-complex vitamins and reduces phytates while sprouting enhances mineral bioavailability and protein digestibility (Zhang *et al.*, 2022). Toasting improves flavor while inactivating enzymes and microbial contaminants, making it an essential step in preparing complementary foods (Lawa *et al.*, 2019). Combining these techniques with locally sourced ingredients can produce complementary foods that are both nutrient-dense and palatable, ensuring better acceptance among children. Yellow maize, sorghum, and millet are carbohydrate-rich cereals with moderate protein content (7-12%) and are good sources of minerals, B vitamins, and antioxidants like carotenoids and phenolic compounds (Taylor and Kruger, 2019; Rouf Shah *et al.*, 2016). Soybeans and walnuts provide high-quality protein (up to 40% in soybeans), essential fatty acids, and bioactive compounds such as isoflavones and polyphenols, while pumpkin seeds are rich in protein (19-25%), magnesium, zinc, and antioxidants. (Messina, 2016; Ros, 2010) Dates are naturally high in sugars, dietary fiber, potassium, and polyphenolic antioxidants, making them a nutrient-dense fruit with small amounts of B vitamins and magnesium (Barakat and Alfheaid, 2023). Date palm fruits have minimal antinutrients, with low levels of tannins that are generally non-problematic (Siddan *et al.*, 2020). Pumpkin seeds, walnuts, and soybeans contain phytates, with walnuts having relatively low levels and soybeans also containing trypsin inhibitors that affect protein digestion and mineral bioavailability (Albuquerque *et al.*, 2020;

Chauhan *et al.*, 2022). Cereals like millet, sorghum, and yellow maize have antinutrients such as phytates, polyphenols, tannins, and oxalates, which can hinder the absorption of key minerals like iron and zinc (Nuss and Tanumihardjo 2010; Gwekweet *et al.*, 2024). The aim of this study is to conduct a comparative analysis of complementary foods formulated from yellow maize, sorghum, millet, and soybeans, fortified with walnuts, pumpkin seeds, and date palm fruits. It will evaluate the macronutrient, micronutrient, and anti-nutritional profiles of these formulations to determine their potential to meet the dietary needs of infants and young children. Comparing the nutrient and antinutrient profiles of the formulated foods will help to identify the nutritional strengths and weaknesses of each food, aiding in the selection of raw materials for balanced diets and food fortification. It will show differences in protein, vitamins, and minerals enabling targeted dietary planning for children aged 1 to 3 while understanding antinutrient levels will guide in the choice of processing methods to reduce their impact and enhance nutrient bioavailability. The findings will support the development of affordable, nutrient-rich complementary foods that will address malnutrition and promote both local economies and sustainable community nutrition.

2. MATERIAL AND METHODS

2.1 Sources of material

The matured raw materials (yellow corn, sorghum, millet, soybeans, walnuts, pumpkin seeds, and date palm fruits) were obtained from the Grain Seed Market in Onitsha, Anambra State. All chemicals and reagents used were of analytical grade manufactured by Sigma-Aldrich (Merck KGaA) and sourced from reputable scientific chemical suppliers at Bridgehead Market, Onitsha, Anambra State.

2.2 Preparation of samples

2.2.1 Preparation of the yellow maize, millet, sorghum, and soybeans

The samples were individually sorted, floated to remove broken grains and foreign materials, and thoroughly washed. Each grain and seed were soaked separately for 48 hours at 30°C to ferment. It was an anaerobic, solid-state fermentation with *Lactobacillus plantarum* and *Saccharomyces cerevisiae* microorganisms responsible. After fermentation, they were drained, rinsed, and placed in a colander covered with muslin cloth, and kept at 25°C. The grains and seeds were rinsed under running water every 12 hours for three days to encourage sprouting. On the fourth day, they were rinsed again, spread on a metal tray, and dried in an oven at 65°C for 12 hours. After oven drying, the soybeans were toasted at 175°C for 10 minutes and cracked to remove the seed coat. An electric milling machine's parts were detached, washed with mild soap and warm water, rinsed thoroughly, and reassembled. The machine was run for three minutes at high speed without any raw material inside to sterilize the internal parts. After that, dried grains and toasted seeds were ground into flour and stored in airtight containers until formulation.

2.2.2 Preparation of the pumpkin seeds, walnut, and date palm fruit

The pumpkin was washed, cut open, and the seeds were scooped out. Any bad seeds were discarded. The seeds were oven-dried at 65°C for 12 hours, then ground into flour using the washed and sterilized electric milling machine. The flour was stored in an airtight container until formulation. Walnut seeds were sorted, and spoilt seeds were removed. It was thoroughly washed, boiled for 30 minutes on an electric stove, de-shelled, and chopped into small pieces. They were blanched at 100°C for 5 minutes, drained, spread on a metal tray,

and oven-dried at 65°C for 12 hours. After drying, they were ground into flour and stored in an airtight container for formulation. Dry date palm fruits were sorted, washed, and cut open to remove the seeds. The fleshy part was oven-dried at 65°C for 6 hours and stored in an airtight container until formulation.

2.3 Formulation of the Complementary Food

The processed raw materials were entered into the NutriSurvey²⁰⁰⁷ software to determine the nutrient composition of the food plan. To achieve an optimized diet, the FAO/WHO recommendations for children aged 12–23 months were applied using the software's linear programming module, with minor adjustments made to the plan. It is important to note that the nutrient values in the database are not always accurate, as they are often approximated and influenced by factors such as plant variety, climate, and processing methods. Three Complementary Food Formulations (CFF) were developed. CFF1 comprises fermented/sprouted yellow maize, fermented/sprouted sorghum, fermented/sprouted millet, fermented/sprouted/toasted soybeans, boiled/blanched/toasted walnuts, and toasted date palm fruits. CFF2 comprises fermented/sprouted yellow maize, fermented/sprouted sorghum, fermented/sprouted millet, fermented/sprouted/toasted soybeans, toasted pumpkin seeds and toasted date palm fruits. CFF3 comprises fermented/sprouted yellow maize, fermented/sprouted sorghum, fermented/sprouted millet, fermented/sprouted/toasted soybeans, boiled/blanched/toasted walnuts, toasted pumpkin seeds, and toasted date palm fruits. Cerelac a commercially available infant complementary food, served as the control.

Table 1: Weight of processed raw materials for CFF

Processed raw materials(g)	CFF1	CFF2	CFF3
Fermented/sprouted yellow maize	25	25	25
Fermented/sprouted sorghum	10	10	10
Fermented/sprouted millet	10	15	12
Fermented/sprouted/toasted soybeans	25	20	23
Boiled/blanched/toasted walnuts	10	-	5
Toasted pumpkin seeds	-	10	5
Toasted date palm fruits	20	20	20
Total	100	100	100

CFF1=25 g yellowmaize:10 g sorghum:10 g millet:25 g soybeans:10 g walnut:0 g pumpkin, 20 g dateplam fruits, CFF2=25 g yellowmaize:10 g sorghum:15 g millet:20 g soybeans:0 g walnut:10 g pumpkin:20 g dateplam fruits andCFF3=25 g yellowmaize:10 g sorghum:12 g millet:23 g soybeans:5 g walnut:5 g pumpkin:20 g dateplam fruits.

Table 2 Cost Analysis of the formulated food

Processed raw materials	Price per 1000 g (₦)	Weight in CFF1 (g)	Cost (₦)	Weight in CFF2 (g)	Cost (₦)	Weight in CFF3 (g)	Cost (₦)
Yellow maize	1000	25	25	25	25	25	25
Sorghum	1500	10	15	10	15	10	15
Millet	1500	10	15	15	25	12	20
Soybean	2000	25	50	20	40	23	50
Walnuts	2500	10	25	-	-	5	15
Pumpkin seeds	2500	-	-	10	25	5	15
Date palm fruits	3000	20	600	20	600	20	600
Total	14000	100	730	100	730	100	740

2.4 Analysis

2.4.1 Proximate analysis

The analysis of crude fiber, crude fat, crude protein, moisture, ash, carbohydrate, and energy contents was conducted using the standard methods established by the AOAC (2019).

2.4.1.1 Moisture contentdetermination:

An empty petri dish was dried in an oven for 10 minutes, cooled in a desiccator for 20 minutes and weighed (w_1). The samples (2 g each) were weighed into the petri dish (w_2) and placed in an oven at 105°C for six hours. They were brought out, cooled in a desiccator, and weighed (w_3). The procedure was repeated three times and a constant weight was obtained. The loss in weight was calculated as the percentage moisture content:

$$\% \text{ Moisture} = \frac{w_2 - w_3}{w_2 - w_1} \times 100$$

Where w_1 = weight of empty petri dish, w_2 = weight of petri dish + sample, w_3 = weight of dried sample + petri dish.

2.4.1.2 Ashcontentdetermination

The ash content was determined using the dry ashing method. An empty crucible was fire-polished in a muffle furnace, allowed to cool in a desiccator for 20 minutes and was weighed (w_1). The samples (2g each) were weighed into crucibles (w_2), transferred into the furnace, and heated at 550°C for 5 hours until the samples were completely ash. The crucibles were removed, and a drop of water was added to expose the unashed portion. The crucibles were placed back in the furnace and heated for more 30 minutes. After this, they were removed, allowed to cool in a desiccator and weighed (w_3). The percentage ash was calculated as:

$$\% \text{ Ash} = \frac{w_3 - w_1}{w_2 - w_1} \times 100$$

Where w_1 = weight of empty crucible, w_2 = weight of crucible + sample, w_3 = weight of crucible + ash.

2.4.1.3 Crude fiber content determination

The crude fiber content was determined using the gravimetric method. Each sample (2 g) was refluxed for 30 minutes with 200 mL of a solution containing 1.25 mL of H_2SO_4 per 100 mL. The mixture was then filtered using a linen cloth, and the residue was washed with boiling water until the washings were neutral. The residue was transferred to a beaker and boiled for another 30 minutes with 200 mL of a solution containing 1.25 g of carbonate-free NaOH per 100 mL. After boiling, the residue was filtered through a thin pad of ignited asbestos and washed into a Gooch crucible. The residue was dried in an oven at 105°C for 3 hours, then cooled in a desiccator and weighed. It was then incinerated in a muffle furnace at 550°C for 3 hours. After cooling the crucible in a desiccator for 20 minutes, the ash was weighed. The percentage of crude fiber was calculated from the weight loss after incineration using the formula:

$$\% \text{ Crude fibre} = \frac{\text{weight of fibre}}{\text{weight of sample}} \times 100$$

2.4.1.4 Crude fat content determination

The determination of crude fat content was by Soxhlet extraction method. Approximately 5 g of the sample (w) was weighed and placed in a thimble made of filter paper and was inserted into the Soxhlet extractor column. A clean, dry round-bottom flask was weighed (w_1) and attached to a Soxhlet extraction unit. Approximately 350 mL of n-hexane was poured into the round-bottom flask, which was then fitted into the Soxhlet apparatus. The flask was heated on an electrothermal heater at 60°C . The solvent evaporated during heating was condensed and refluxed back into the thimble by the condenser, ensuring continuous extraction of fat from the sample. The solvent-fat mixture flows back into the flask, and the process was repeated continuously for 8 hours until complete fat extraction. After the extraction was complete, the thimble was removed, and the solvent was recovered by distillation. The flask, now containing only the extracted fat, was placed on a water bath to remove any residual solvent. It was then transferred to an oven at 105°C for 2 hours to ensure complete drying. After drying, the flask was cooled in a desiccator and reweighed (w_2).

The crude fat content was calculated using the formula:

$$\% \text{ Crude fat} = \frac{w_2 - w_1}{w} \times 100$$

Where w = weight of sample, w_1 = weight of round bottom flask and w_2 = weight of flask + residue.

2.4.1.5 Carbohydrate content determination

The carbohydrate content was determined by difference, using the formula:

$$\text{Carbohydrate (\%)} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Crude Fibre} + \% \text{ Crude Protein} + \% \text{ Fat})$$

2.4.1.6 Energy Content Determination

The energy content was calculated using the Atwater factor. The values of 4 kcal/g for protein and carbohydrate and 9 kcal/g for fat are used. The total energy was estimated as:

$$\text{Energy (kcal)} = (\% \text{ Protein}) + (\% \text{ Carbohydrate}) + (\% \text{ Fat})$$

2.4.2 Mineral analysis

The method described by Souza *et al.* (2020) was used with little modification. The samples were dried in an oven at 105°C for 8 hours to constant weight. They were cooled in a desiccator for 20 minutes and ground into fine powder using an electric milling machine. Equivalent 10 mL of 65% concentrated nitric acid HNO₃ was added to 1 g of the powdered sample in a digestion flask. The sample was allowed to pre-digest in a fume cupboard at room temperature for 30 minutes to reduce the risk of violent reactions during heating. After 30 minutes, the sample was heated at 140°C using a heating mantle until the solution becomes nearly clear. To ensure complete digestion, 5 mL of 30 % hydrogen peroxide H₂O₂ was added 1 mL at a time after cooling the initial digested solution followed by reheating, until the solution became clear. Finally, the digested sample was cooled transferred to 100 mL volumetric flask and diluted to the mark with distilled water. The resulting solution was filtered into a second 100 mL volumetric flask and brought to volume with distilled water. A series of standard metal solutions covering the optimal concentration range for each mineral were prepared by diluting stock solutions with water containing 1.5 mL of concentrated nitric acid. A calibration blank, containing all reagents except mineral standards, was also prepared. Calibration of the AAS was performed using standard solutions of the target elements, ensuring accurate wavelength and lamp settings for each element. A calibration curve for each mineral was generated by plotting the absorbance of the standards against their concentrations, providing a reference for determining mineral content in the flour samples. Samples were aspirated into the AAS, absorbances were measured, compared to the calibration curve and mineral concentrations were determined.

2.4.3 Vitamin analysis

2.4.3.1 Determination of vitamin A

Vitamin A was determined following the procedure of Kesuma *et al.* (2020) with little modification. Each one gram sample was mixed with 1.0 mL of a saponification mixture, prepared by dissolving 12 grams of potassium hydroxide in 88 mL of ethanol. The mixture was then refluxed at 60°C for 20 minutes in the dark to prevent any interference from light. After saponification, the mixture was transferred to boiling tubes and allowed to cool. Subsequently, 20 mL of water was added, and the solution was thoroughly mixed. Vitamin A was extracted twice with 10 mL portions of petroleum ether at 40°C. The extracts were then cooled and washed with water to remove impurities, and anhydrous sodium sulfate was added to eliminate any residual moisture. A 1.0 mL aliquot of the sample extract was taken and evaporated to dryness at 60°C. The residue was dissolved in 1.0 mL of chloroform. Standards containing vitamin A palmitate at concentrations ranging from 0 to 7.5 mg were pipetted into a series of test tubes, with each volume made up to 1.0 mL using chloroform. To each standard and sample tube, 2.0 mL of tricarboxylic acid (TCA) reagent was added rapidly, mixed thoroughly, and the absorbance was measured immediately at 620 nm using a Genesys 10UV spectrophotometer. The concentration of vitamin A in each sample was determined based on its absorbance. Vitamin A content was obtained in mg/kg and converted to µg/100g.

2.4.3.2 Determination of vitamin D

Vitamin D was assayed following the method by Vinhas *et al.* (2017). A 25 mg vitamin D working standard was weighed and placed into a 25 mL volumetric flask. It was dissolved in a solution mixture of chloroform and methanol (1:9 ratio), diluted with the same solution, and made up to the mark. The mixture was thoroughly mixed. Each flour sample (0.1 g) was weighed and placed into a 25 mL volumetric flask. This sample was dissolved in the chloroform and methanol mixture (1:9 ratio), diluted to the mark, and mixed thoroughly. Subsequently, 1.6 mL of 0.25 M HCl, 0.5 mL of 15% trichloroacetic acid, and 0.5 mL of 0.375% thiobarbituric acid (TBA) were added. The mixture was transferred to a cuvette and allowed to develop for 30 minutes. Its absorbance was measured at 464 nm against a blank using spectrophotometer. Vitamin D content was obtained in $\mu\text{g}/\text{kg}$ and converted to $\mu\text{g}/100\text{g}$

2.4.3.3 Determination of Vitamin E

The method described by Kesuma *et al.* (2020) was used to estimate vitamin E. Each sample (2.5 grams) was homogenized in 50 mL of 0.1 M sulfuric acid and left to stand overnight. The contents were shaken vigorously and filtered using Whatman No. 1 filter paper. Aliquots of the resulting filtrate were taken for the estimation. Equivalent 1.5 mL of the sample filtrate, 1.5 mL of a vitamin E standard, and 1.5 mL of water were each pipetted into separate stoppered centrifuge tubes. Ethanol (1.5 mL) and xylene (1.5 mL) were then added to each tube, mixed thoroughly, and centrifuged. A 1.0 mL portion of the xylene layer was transferred to a new stoppered tube, and 1.0 mL of dipyrindyl reagent was added, followed by thorough mixing. Finally, 1.5 mL of this mixture was transferred into a cuvette, and the absorbance was measured at 460 nm. Vitamin E content was obtained in mg/kg and converted to $\text{mg}/100\text{g}$

2.4.3.4 Determination of Vitamins B1 and B2 (Thiamine and Riboflavin)

One gram of each sample was weighed into a conical flask and dissolved in 100 mL of deionized water. The mixture was thoroughly shaken, heated for 5 minutes, allowed to cool, and filtered. The resulting filtrate was poured into a cuvette, and the absorbance was measured at the respective wavelengths for the vitamins (261 nm for vitamin B1 and 242 nm for vitamin B2) using a spectrophotometer. The concentration ($\text{mg } \%$) was calculated using the formula:

$$\text{Concentration (mg \%)} = \frac{A \times D.F \times \text{volume of cuvette}}{E}$$

where A = absorbance, E = extinction coefficient = 25 for B₁ and B₂, D.F = dilution factor.

2.4.3.5 Determination of vitamin B3(Niacin)

Five grams of each sample were dissolved in 20 mL of anhydrous glacial acetic acid and gently warmed. Then, 5 mL of acetic anhydride was added and thoroughly mixed. Two drops of crystal violet solution were introduced as an indicator. The mixture was titrated with 0.1M perchloric acid until a greenish-blue color was observed. The vitamin B3 content ($\text{mg } \%$) was calculated using the formula:

$$\text{Vitamin B}_3 = \frac{\text{titre value} \times 0.0122}{0.1}$$

2.4.3.6 Determination of Vitamin B6 (Pyridoxine)

Five grams of each sample were dissolved in a mixture of 5 mL of anhydrous glacial acetic acid and 6 mL of 0.1M mercury (II) acetate solution. Two drops of crystal violet solution were added as an indicator. The mixture was titrated with 0.1M perchloric acid until a green color endpoint was reached.

For calculation: each cm^3 of 0.1M perchloric acid is equivalent to 0.02056 g of $\text{C}_8\text{H}_{11}\text{NO}_3\text{HCl}$.

It was expressed in mg/100g

2.4.3.7 Determination of Vitamin B12(Cobalamin)

Approximately 0.1 g of each sample was weighed and placed in a separator. Then, 5 mL of water was added, mixed thoroughly, and extracted with 5 mL of chloroform. The aqueous layer was discarded, and the chloroform layer was passed through anhydrous sodium sulfate into a dry 50 mL volumetric flask, then diluted to 50 mL with chloroform. A 2 mL aliquot of the extracted sample, along with a blank solution, was transferred into test tubes. To each test tube, 2 mL of a 0.2 % phenyl hydrazine solution (prepared in hydrochloric acid and alcohol in a 1:5 v/v ratio) was added and mixed thoroughly. The mixture was heated in a water bath until nearly dry, then allowed to cool to room temperature. Next, a 2 mL solution of ammonia and alcohol (1:1 ratio) and 1 mL of pyridine were added to each test tube. The absorbance was measured at 635 nm against the blank. Standard cobalamin was prepared and analyzed in the same way as the samples. A calibration curve was constructed, and the sample concentrations were determined by extrapolation. It was obtained in mg/kg and converted to $\mu\text{g}/100\text{g}$

2.4.3.8 Determination of vitamin C

The spectrophotometric method described by Shara *et al.* (2019) was utilized to estimate vitamin C. Ascorbate was extracted from a 1.0 g sample using 4% TCA, and the volume was adjusted to 10 mL with the same TCA. After centrifuging the mixture at 2000 rpm for 10 minutes, the supernatant was treated with a pinch of activated charcoal, vigorously shaken with a cyclomixer, and allowed to stand for 5 minutes. The charcoal particles were then removed by centrifugation, and aliquots of the filtrate were used for estimation. Standard ascorbate solutions ranging from 0.2 to 1.0 mL, along with 0.5 mL and 1.0 mL aliquots of the supernatant, were taken in test tubes. The volume in each tube was brought up to 2.0 mL with 4% TCA. Then, 0.5 mL of dinitrophenyl hydrazine (DNPH) reagent was added to each tube, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37°C for 3 hours, resulting in the formation of osazone crystals. These crystals were dissolved in 2.5 mL of 85% sulfuric acid. For the blank sample, DNPH reagent and thiourea were added after the addition of sulfuric acid. The tubes were then cooled in ice, and the absorbance was read at 540 nm using a spectrophotometer. A standard graph was constructed using an electronic calculator set to linear regression mode. The concentration of ascorbate in the samples was calculated and expressed in mg/kg using the formula ($Y = mx + c$), where Y = is the absorbance, x = is the concentration, m = is 0.0135, and c = is 0.0062

2.4.4 Determination of anti-nutrients

2.4.4.1 Determination of condensed tannin content

The method described by Asres *et al.* (2018) was modified. The crushed sample (20 g) was placed in a conical flask, and 100 mL of petroleum ether was added. The flask was covered and allowed to stand for 24 hours. After this, the sample was filtered, and the residue was left to stand for 15 minutes to allow the petroleum ether to evaporate. The residue was re-extracted by soaking in 100 mL of 10% acetic acid in ethanol for 4 hours. The sample was filtered, and the filtrate was collected. To precipitate the alkaloids, 25 mL of NH_4OH was added to the filtrate. The mixture was heated on an electric hot plate for to remove excess NH_4OH . From the remaining volume 5 mL was taken for analysis. Ethanol (20 mL) was added to the 5 mL aliquot, and the solution was titrated with 0.1 M NaOH, using 2 drops of phenolphthalein as an indicator until a pink endpoint was reached. The tannin content was calculated as a percentage of the sample's molarity using the formula:

$$\% \text{ Tannic acid content} = \frac{C_1 \times 100}{\text{weight of sample analysed}}$$

where C_1 = conc. of tannic acid

2.4.4.2 Determination of phytate content

Phytate content was measured following the method of Asres *et al.* (2018) with some modifications. A 0.2 g sample was soaked in 100 mL of 2% HCl for 3 hours and then filtered. The filtrate (50 mL) was diluted with 100 mL of distilled water, and 10 mL of 0.3% ammonium thiocyanate indicator was added. The solution was then titrated with a standard iron III chloride solution containing 0.00195g iron per mL. The phytic acid concentration was calculated using the formula

$$\text{Phytic acid} = \frac{\text{titre value} \times 0.00195 \times 1.19 \times 100}{\text{weight of sample}}$$

2.4.4.3 Determination of oxalate content

The method described by Gemede (2020) was modified. A 2g sample was suspended in 190 mL of distilled water in a 250 mL volumetric flask, and 10 mL of 6M HCl was added. This mixture was digested at 100°C for 1 hour, cooled, and brought to volume with distilled water before filtration. A 125 mL portions of the filtrate were measured into beakers, and 4 drops of methyl red indicator were added. Ammonium hydroxide solution was added dropwise until the solution turned from salmon pink to faint yellow (pH 4-4.5). Each portion was heated to 90°C, cooled, and filtered to remove ferrous ion precipitate. The filtrate was reheated to 90°C, and 10 mL of 5% CaCl_2 solution was added with constant stirring. After heating, the solution was cooled and left overnight at room temperature. It was then centrifuged at 2500 rpm for 5 minutes, the supernatant decanted, and the precipitate dissolved in 10 mL of 20% H_2SO_4 . The resulting solution from the 2 g sample was brought to a final volume of 300 mL. A 125 mL aliquot was heated to near boiling and titrated with standardized 0.05M KMnO_4 to a faint pink endpoint persisting for 30 seconds. Calcium oxalate content was calculated using the provided formula

$$\text{Calcium oxalate content} = \frac{T \times V_{me} \times DF \times 10^5}{ME \times M_f} \text{ (mg/100g)}$$

where T = titre value, Vme = volume-mass equivalent, DF = dilution factor = 2.4, ME = molar equivalent of KMnO_4 in oxalate and Mf = mass of sample used

2.4.4.5 Determination of cardiac glycosides content

1 mL of extract was mixed with 1 mL of 2% 3,5-dinitrosalicylic acid in methanol and 1 mL of 5% aqueous NaOH. This mixture was boiled for two minutes until a brick-red precipitate formed. The precipitate was filtered using pre-weighed filter paper, dried at 50°C , and weighed again.

The cardiac glycoside was calculated using the formula

$$\% \text{ Cardiac glycoside} = \frac{(\text{weight of filter paper} + \text{residue}) - (\text{weight of filter paper}) \times 100}{\text{weight of sample analysed}}$$

2.4.4.6 Determination of phenol content

Phenol content was determined using the spectrophotometry method. The sample (5 g) was boiled with 50 mL of diethyl ether for 15 minutes. A 5 mL aliquot of the boiled sample was transferred to a 50 mL flask and diluted with 10 mL of distilled water. A 2 mL of ammonium hydroxide and 5 mL of concentrated pentanol were added. The mixture was brought to volume, allowed to react for 30 minutes for color development, and the absorbance measured at 505 nm.

2.4.4.7 Determination of heamagglutinin content

A 20 mL of 0.9% Sodium chloride was added to 2 g of the sample and the suspension shaken vigorously for 1 minute. The supernatant was left to stand for 1 hour and then centrifuged at 2000 rpm for 10 minutes. The suspension was filtered. The supernatant was collected and used as crude agglutination extract. The absorbance was read at 420 nm.

$$\text{Conc of sample (mg/l)} = \frac{\text{Asorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

2.4.4.8 Determination of cynogenicglycoside

Acid titration method was used. Ten grams of sample, ground to pass N0.20 sieve, was placed in 800 mLkjeldahl flask. Water (100 mL) was added to the sample and was macerated at room temperature for 2 hours. Equivalent 100 mL of H_2O was added, and the tip of the condenser was dipped below the surface of the liquid in receiver flask. It was steam distilled and the distillate collected in 20 mL of 0.02 M AgNO_3 acidified with 1 mL of HNO_3 . When 150 mL has passed over, the distillate was filtered through Büchner funnel and washed with 50 mL of H_2O . Excess AgNO_3 in combined filtrate and washings was titrated with 0.02 M KCN, using Fe alum indicator.

$$1\text{cm}^3 \text{ 0.02M AgNO}_3 = 0.54 \text{ mg HCN}$$

2.5 Statistical Analysis

The results were reported as the mean \pm standard deviation from duplicate experiments. The statistical significance of the data was evaluated using one-way analysis of variance (ANOVA). OriginPro 2024 software was used to identify significant differences. The Tukey test was used to separate significant means, with differences considered significant at ($p < 0.05$)

3. RESULTS AND DISCUSSION

Table 3 shows the proximate composition of cerelac and Complementary Food Formulations (CFF1, CFF2, and CFF3). The result reveals significant ($p < 0.05$) differences in nutrient composition between cerelac and CFF, with some parameters aligning with WHO recommended nutrient intake (RNI) guidelines for complementary foods. The crude fiber content is significantly higher ($p < 0.05$) in complementary food formulations (4.31–4.56%) across CFF1 to CFF3 compared to cerelac (3.01%), indicating a nutritional advantage for digestive health. Similarly, crude fat is slightly higher in CFF (5.43–5.52%) than in cerelac (4.62%), meeting the World Health Organization Recommended Nutrient Intake WHO RNI of 4–8% for infant foods. Crude fat in CFF is consistent with findings from Keyata *et al.* (2021), which reported fat content of 4–8% in enriched complementary blends, supporting energy density and nutrient absorption. This is consistent with the findings from Afolabi *et al.* (2017), on the importance of maintaining fat content within a moderate range for energy density in weaning foods. Protein content in CFF ranges from 10.30% (CFF1) to 11.80% (CFF3), comparable to cerelac's 11.90%, aligning with WHO's guideline of 10–15% for adequate growth. The crude protein content of 11.45% reported for fermented maize, toasted soybean complementary food by Ademulegun *et al.* (2021), is close to 11.67% obtained for CFF2. Also, the protein content of CFF aligns well with studies that emphasize protein levels of 10–15% as optimal for complementary feeding (Akinola *et al.*, 2017). There is no significant difference ($p < 0.05$) in moisture content across all samples ranging from 10.50% in cerelac to 10.91% in CFF1, falling within the acceptable range for complementary foods to ensure shelf stability. The ash content, an indicator of mineral presence, is higher in CFF (3.40–3.44%) compared to cerelac (2.46%), suggesting better mineral availability. The higher ash content in CFF aligns with studies suggesting that local blends often incorporate mineral-rich ingredients, offering enhanced micronutrient density. Carbohydrate content oscillated from (64.05 - 67.75) % in CFF1 and CFF3 respectively which aligns with cerelac's (67.53%). High carbohydrate content is often desired in infant foods for sustained energy (Adegunwa *et al.*, 2017). The carbohydrate content of CFF2 (64.86%) is close to 64.90% obtained for SAPO1 (soybean, amaranth grains, pumpkin seeds, orange fleshed sweet potatoes) complementary food reported by Marcel (2022). However, carbohydrate content in cerelac surpasses that in CFF contributing to slightly higher energy values in cerelac (359.27 kcal) compared to CFF (353.04–354.94 kcal). Energy values for both cerelac and CFF are in line with (355.37–384.39 kcal) reported by Tanyitiku and Petcheu (2022), for processed sorghum, soybeans, mango complementary foods, meeting the WHO's recommendation of 350–400 kcal/100 g for complementary foods. The energy content of CFF agrees with 352.0 kcal obtained by Falmata *et al.* (2014) for complementary weaning food formulated from sprouted/fermented sorghum fortified with cowpea and ground nut.

Table 3 Proximate composition of Cerelac and CFF

Parameters (%)	Cerelac	CFF1	CFF2	CFF3
Crude fibre	3.01 ^b ± 0.00	4.31 ^{ab} ± 0.22	4.41 ^a ± 0.36	4.56 ^a ± 0.54
Crude fat	4.62 ^a ± 0.47	5.52 ^a ± 0.38	5.43 ^a ± 0.16	5.44 ^a ± 0.39
Crude protein	11.90 ^a ± 0.50	10.30 ^b ± 0.07	11.67 ^{ab} ± 0.19	11.80 ^a ± 0.50
Moisture	10.50 ^a ± 0.25	10.91 ^a ± 0.06	10.84 ^a ± 0.98	10.53 ^a ± 0.17
Ash	2.46 ^a ± 0.64	3.42 ^a ± 0.11	3.40 ^a ± 0.15	3.44 ^a ± 0.62
Carbohydrate	67.53 ^a ± 1.36	64.05 ^b ± 0.48	64.86 ^{ab} ± 0.04	65.75 ^{ab} ± 0.22
Energy (kcal)	359.27 ^a ± 0.76	353.04 ^a ± 3.47	354.94 ^a ± 0.53	353.11 ^a ± 2.32

Values are presented in the form of mean of duplicate determinations ± standard deviation. Means with different superscripts in the same row are significantly ($p < 0.05$) different.

CFF1 = 25 g yellow maize:10 g sorghum:10 g millet:25 g soybeans:10 g walnut:0 g pumpkin, 20 g date plam fruits.

CFF2 = 25 g yellow maize:10 g sorghum:15 g millet:20 g soybeans:0 g walnut:10 g pumpkin:20 g date plam fruits.

CFF3 = 25 g yellow maize:10 g sorghum:12 g millet:23 g soybeans:5 g walnut:5 g pumpkin:20 g date plam fruits.

The mineral composition of cerelac and CFF as presented in Table 4 demonstrates significant ($p < 0.05$) differences, with several parameters meeting the WHO RNI for infants. Sodium levels in both cerelac (79.08 mg/100 g) and CFF (78.39–83.65 mg/100 g) in CFF2 and CFF1 are comparable, and within acceptable ranges for complementary foods. The sodium level aligns with Akinola *et al.* (2020), who reported similar sodium content in fortified complementary foods, indicating adequate seasoning for palatability. Calcium content in CFF (58.16–69.13 mg/100 g) in CFF1 and CFF2, surpasses cerelac (48.83 mg/100 g) both significantly ($p < 0.05$) fall short of the WHO RNI of 500 mg/day. This suggests the need for additional dietary sources of calcium to meet daily requirements and echoes findings by Adegunwa *et al.* (2017), where traditional formulations often lacked sufficient calcium, necessitating fortification with calcium-rich ingredients. Potassium levels in all samples align well with WHO RNI of 15 µg/day. Potassium content is higher in CFF2 (75.86 mg/100 g) compared to cerelac (61.42 mg/100 g), which supports the importance of potassium in fluid balance and muscle function. Magnesium content in CFF (62.82–73.33) mg/100 g in CFF3 and CFF2 meets and exceeds the WHO RNI of 60 mg/day, outperforming cerelac (59.39 mg/100 g), which barely meets the minimum. Ijarotimi *et al.* (2022), emphasized that magnesium fortification is critical in preventing deficiencies. Phosphorus level in CFF3 (225.86 mg/100 g) is significantly ($p < 0.05$) higher than in cerelac (166.71 mg/100 g), aligning with Abeshu *et al.* (2016), who found that indigenous formulations often contained superior phosphorus levels due to natural ingredient choices. Iron contents vary with CFF2 (11.70 mg/100 g) exceeding cerelac (6.62 mg/100 g) and meeting WHO's range of 3.9–11.6 mg/day, critical for preventing anemia. Zinc levels in both cerelac (7.70 mg/100 g) and CFF (4.77–7.71) mg/100 g in CFF1 and CFF3 are adequate according to the RNI (2.4–8.3 mg/day). Zinc is a critical nutrient for immunity in infants. Manganese and iodine levels in all samples, including cerelac, align well with RNI, showing balanced fortification strategies.

Table 4 Mineral Composition of CFF and cerelac

Parameters (mg/100g)	Cerelac	CFF1	CFF2	CFF3
Sodium	79.08 ^a ± 0.64	83.65 ^a ± 2.26	78.39 ^a ± 1.29	83.52 ^a ± 3.96
Calcium	48.83 ^c ± 0.15	58.16 ^b ± 1.09	69.13 ^a ± 0.28	58.16 ^b ± 1.10
Potassium	61.42 ^a ± 0.74	63.94 ^a ± 7.07	75.86 ^a ± 9.79	60.83 ^a ± 1.41
Magnesium	59.39 ^c ± 0.64	63.72 ^b ± 0.45	73.33 ^a ± 0.77	62.82 ^b ± 0.05
Phosphorous	166.71 ^d ± 0.76	208.04 ^b ± 1.59	185.06 ^c ± 5.75	225.86 ^a ± 4.09
Iron	6.62 ^b ± 2.55	4.98 ^b ± 0.64	11.70 ^a ± 1.91	4.75 ^b ± 0.99
Zinc	7.70 ^a ± 2.17	4.77 ^a ± 4.77	6.35 ^a ± 1.72	7.71 ^a ± 2.17
Manganese	3.60 ^a ± 0.06	3.27 ^b ± 0.06	1.93 ^c ± 0.06	3.24 ^b ± 0.07
Iodine	67.11 ^a ± 0.07	74.46 ^a ± 0.25	74.26 ^a ± 0.61	72.83 ^a ± 0.07

Values are presented in the form of mean of duplicate determinations ± standard deviation. Means with different superscripts in the same row are significantly ($p < 0.05$) different.

The vitamin content in CFF and cerelac is presented in Table 5. It reveals significant ($p < 0.05$) differences, with varying adherence to WHO (RNI). Vitamin A levels are comparable across cerelac (509.96 µg/100 g) and CFF (502.38–525.99) µg/100 g in CFF2 and CFF3, all exceeding the WHO RNI of 400 µg/RE. This is consistent with Akinola *et al.* (2020), who reported fortified blends exceeding vitamin A requirements for visual and immune health. Vitamin C content is significantly ($p < 0.05$) higher in cerelac (31.30 mg/100 g), meeting the WHO RNI of 30 mg/day, whereas CFF (2.39–2.79) mg/100 g in CFF2 and CFF1 fall short of the standard. This is consistent with the findings by Akande *et al.* (2023), that local formulations often lack sufficient vitamin C due to limited inclusion of citrus-rich ingredients. Vitamin D levels in cerelac (4.48 µg/100 g) and CFF (4.34–4.70) µg/100 g in CFF1 and CFF2 are close to the WHO RNI of 5 µg/day but remain slightly deficient. This trend is supported by findings from Lavelli *et al.* (2021), where traditional formulations showed suboptimal Vitamin D levels due to inadequate fortification. Vitamin E level is higher in CFF2 (9.47 mg/100 g) than Cerelac (8.77 mg/100 g), but both fall below the WHO RNI of 15 mg/day. This reflects results from Abeshu *et al.* (2016) that highlighted gaps in Vitamin E content in complementary foods, particularly those reliant on non-oil-based fortification. For B-complex vitamins, cerelac (5.18 mg/100 g) is higher in B1 than CFF (0.22–0.26) mg/100 g in CFF1 and CFF2. The vitamin B2 content in cerelac (4.23 mg/100 g) is higher than that of CFF (0.24–0.34) mg/100 g in CFF2 and CFF3. This is consistent with findings from Olapade *et al.* (2018) emphasizing the benefits of commercial fortification processes. Wambui *et al.* (2022), stated that water-soluble vitamins are more unstable when subjected to processing compared to fat-soluble vitamins. Conversely, B12 levels are significantly ($p < 0.05$) higher in CFF2 (4.69 µg/100 g) and CFF3 (4.43 µg/100 g) compared to Cerelac (1.05 µg/100 g), well above the RNI (0.9 µg/day), essential for red blood cell formation. Izuakore *et al.*, (2024) stated that fermentation, sprouting and toasting enriched vitamin B12 content. However, B3 (5.59 mg/100 g) in cerelac and CFF (3.49–3.63) mg/100 g in CFF2 and CFF1, fall below the RNI of 6 mg/day. Similarly, B6 (0.33 mg/100 g) in cerelac and CFF (0.22–0.25) mg/100 g in CFF2 and CFF1 remain insufficient across all samples, reflecting a common shortfall identified in previous studies.

Table 5 Vitamin Composition of CFF and cerelac

Parameters	Cerelac	CFF1	CFF2	CFF3
A (µg/100g)	509.96 ^a ± 1.75	515.81 ^a ± 4.28	502.38 ^a ± 9.74	525.99 ^a ± 16.18
C (mg/100g)	31.30 ^a ± 0.47	2.79 ^b ± 0.06	2.39 ^b ± 0.06	2.48 ^b ± 0.01
D (µg/100g)	4.48 ^{ab} ± 0.04	4.34 ^a ± 0.01	4.70 ^a ± 0.16	4.39 ^{ab} ± 0.03
E (mg/100g)	8.77 ^b ± 0.01	9.02 ^{ab} ± 0.04	9.47 ^a ± 0.20	8.25 ^c ± 0.14
B1 (mg/100g)	5.18 ^a ± 0.03	0.22 ^b ± 0.01	0.26 ^b ± 0.01	0.24 ^b ± 0.01
B2 (mg/100g)	4.23 ^a ± 0.04	0.34 ^b ± 0.01	0.24 ^c ± 0.01	0.29 ^{bc} ± 0
B3 (mg/100g)	5.59 ^a ± 0.03	3.63 ^b ± 0.11	3.49 ^b ± 0.10	3.55 ^b ± 0.01
B6 (mg/100g)	0.33 ^a ± 0.01	0.22 ^c ± 0.01	0.25 ^b ± 0	0.23 ^c ± 0.01
B12 (µg/100g)	1.05 ^c ± 0.04	4.16 ^b ± 0.10	4.69 ^a ± 0.13	4.43 ^{ab} ± 0.04

Values are presented in the form of mean of duplicate determinations ± standard deviation. Means with different superscripts in the same row are significantly ($p < 0.05$) different.

Figure 1 shows the result of anti-nutrient concentrations in the CFF and cerelac. The result of CFF compared to cerelac reveals key insights into their compliance with WHO standards for complementary foods. The CFF exhibit reduced levels of these anti-nutrients compared to cerelac, indicating the efficacy of the processing methods employed, and highlighting their superior nutritional safety. Phytate levels are reduced in the CFF (3.45 - 4.36) mg/g in CFF2 and CFF3 compared to cerelac (4.78 mg/g), aligning with findings by Zang *et al.* (2022), who reported that fermentation and sprouting significantly lower phytate content by activating phytase enzymes. The phytate levels in both cerelac and CFF are below 5 mg/g set by Codex Alimentarius to ensure adequate mineral absorption. Tannin level in the CFF3 (8.99 mg/g) is minimized, comparable to reductions observed in traditional processing methods described by Akinsola *et al.* (2017), where toasting was shown to inactivate tannin-binding proteins. The moderate presence of anthocyanin in CFF (9.32-12.39) mg/g in CFF1 and CFF2 compared to cerelac (23.82 mg/g) aligns with prior studies indicating that controlled processing retains beneficial phytochemicals, which have antioxidant properties (Samtiya *et al.*, 2020). Moreover, the presence of anthocyanins in the CFF, though reduced compared to cerelac, remains within permissible levels and contributes to antioxidant benefits, which WHO recognizes as advantageous for child health when appropriately balanced. The reduced cyanogenic glycosides in CFF1 (1.19 mg/g) compared to cerelac (1.89 mg/g) and hemagglutinin in CFF3 (0.00027 mg/g) compared to cerelac (0.00042 mg/g) indicates the safety improvements made through processing, supporting findings by Adebisi *et al.* (2017). This reduction in anti-nutrient levels enhances the bioavailability of essential minerals like iron and zinc, critical for infant development.

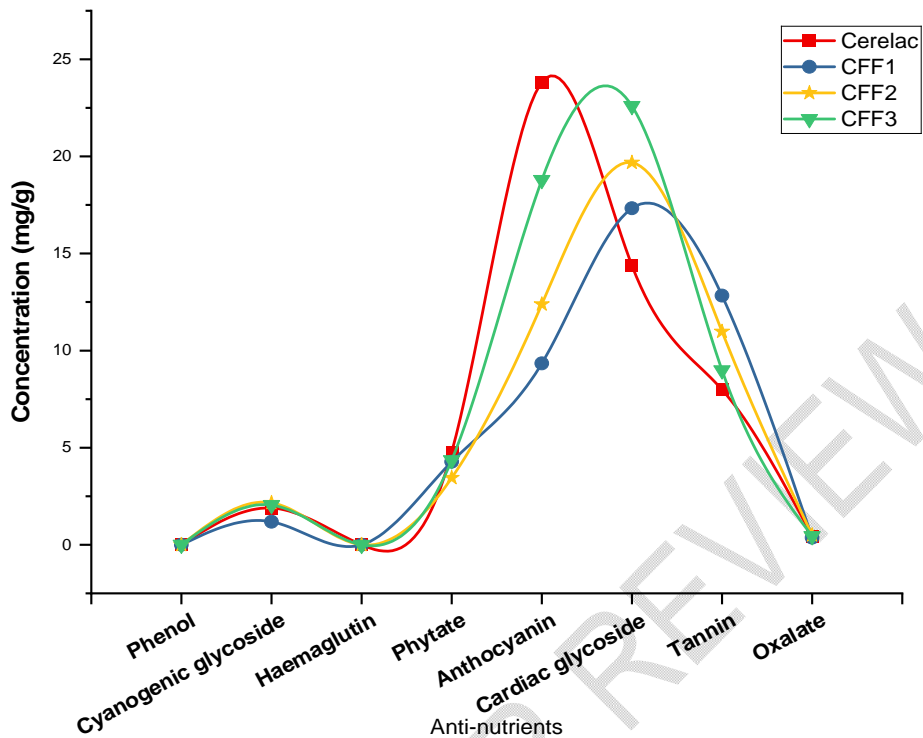


Figure 1 Anti-nutrient concentrations in CFF and cerelac.

4 Conclusion

The findings from this study showed the comparative analysis of nutrient and anti-nutrient profiles in complementary foods formulated from yellow maize, sorghum, millet, and soybeans fortified with walnuts, pumpkin seeds, and date palm fruits. CFF formulations are higher in fiber and ash content than cerelac and met the WHO RNI for energy in complementary foods. CFF2 and CFF3, demonstrate superior content in key minerals like calcium, magnesium, zinc and iron when compared to cerelac. Cerelac surpasses CFF in vitamin C and B vitamins (except B12), while CFF are higher in vitamin A, D and E. The CFF compared to cerelac have lower phytate and anthocyanin anti-nutrient levels. The cost analysis reveals that CFF is cheaper than cerelac. These results reveal significant ($p < 0.05$) differences, with most parameters aligning with WHO (RNI) guidelines for complementary foods making them cheap, nutrient-rich complementary foods that will address malnutrition and promote both local economies and sustainable community nutrition. Further analysis to determine the fatty acid profile, essential amino acids and in-vivo behavioural studies to validate the ability of the CFF to support growth and cognitive development is recommended.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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