

Micronutrients Composition Of A Formulated Plant-based Ready-to-use Supplementary Food (RUSF) Cookies to Address Nutrient Deficiencies in Moderate Acute Malnutrition (MAM), Convalescence And General Health Maintenance

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Abstract: The efficacy of Ready-to-Use Supplementary Foods (RUSF) depends critically on adequate micronutrient bioavailability and consumer acceptability, presenting particular challenges for plant-based formulations. This study comprehensively evaluated the micronutrient composition of a novel plant-based RUSF cookie formulated from indigenous Nigerian ingredients. Two cookie formulations (FPP A for MAM/convalescence; FPP B for general health) were analyzed for vitamin content using HPLC, spectrophotometric methods, and mineral content using Atomic Absorption Spectrophotometry. FPP A demonstrated exceptional mineral content in phosphorus (752.66 ± 1.11 mg/100kcal), potassium (1320.79 ± 0.18 mg/100kcal), and magnesium (213.71 ± 0.68 mg/100kcal), significantly exceeding RUSF standards ($p < 0.05$). However, iron (11.38 ± 0.09 mg/100kcal) and zinc (10.09 ± 0.12 mg/100kcal) contents were slightly below standards. Vitamin analysis revealed adequate levels of thiamine (1.45 ± 0.02 mg/100g), pyridoxine (2.53 ± 0.02 mg/100g), folate (343.41 ± 1.22 mg/100g), vitamins D and E, but significant deficiencies in vitamins A (343.81 ± 0.48 µg/100g), B2 (1.52 ± 0.02 mg/100g), B3 (10.57 ± 0.08 mg/100g), and C (57.83 ± 0.22 mg/100g). The plant-based RUSF cookie demonstrates excellent mineral bioavailability potential and good B-vitamin content but requires strategic fortification to address iron, zinc, and specific vitamin deficiencies.

Keywords: Micronutrients, Bioavailability, RUSF, Plant-based, Food Fortification, Nigeria.

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Introduction

Moderate Acute Malnutrition (MAM) represents a critical public health challenge characterized not only by energy and protein deficits but also by profound micronutrient deficiencies - a condition often termed "hidden hunger" (WHO, 2020). The management of MAM through Ready-to-Use Supplementary Foods (RUSF) requires meticulous attention to micronutrient composition, as vitamins and minerals play essential roles in immune function, linear growth, metabolic recovery, and cognitive development (Bhutta et al., 2013). While plant-based RUSF formulations offer significant advantages in cost-effectiveness, cultural appropriateness, and local sustainability, they present unique challenges regarding micronutrient content and bioavailability (Gibson et al., 2010).

The nutritional efficacy of plant-based therapeutic foods is complimented by the natural presence of micronutrients such as Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B6, Vitamin B9 and Minerals (Hurrell & Egli, 2010). This is particularly important where plant-based foods constitute the primary dietary staple, and where micronutrient deficiencies are already prevalent (Osibogun et al., 2021). Previous attempts to develop plant-based RUSFs in Nigeria have often focused

primarily on macronutrient composition, with limited comprehensive analysis of the complex interplay of micronutrient content. Understanding these contents is essential for developing effective, acceptable, and biologically viable nutritional products that can successfully address the triple burden of malnutrition in resource-limited settings (Lazzerini et al., 2020).

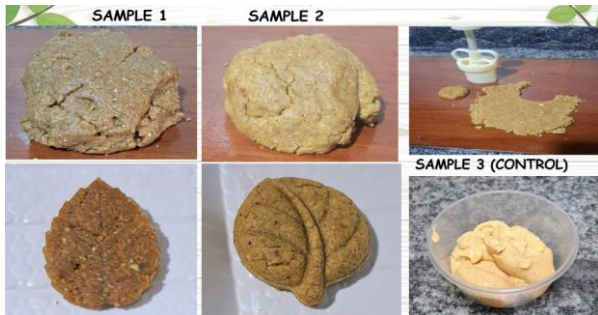
This study therefore aimed to comprehensively analyse the vitamin and mineral composition of a novel plant-based RUSF cookie formulated from indigenous Nigerian ingredients using advanced analytical techniques.

Materials and Methods

Cookie Formulations and Preparation

Two primary cookie formulations were developed based on extensive preliminary research: FPP A, designed as a therapeutic RUSF for MAM and convalescence, and FPP B, formulated for general health maintenance. The formulations were based on optimized composite flours of oat (*Avena sativa*), sesame (*Sesamum indicum*), and baobab (*Adansonia digitata*), supplemented with pumpkin seed flour (*Cucurbita pepo*), peanut butter (*Arachis hypogaea*), and other locally sourced ingredients. The ingredient selection was based on comprehensive nutritional

profiling to ensure optimal nutrient density. A commercial RUSF served as the control (Sample 3) for comparative analysis. All plant materials were authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD) Herbarium, Abuja, with voucher specimens deposited for future reference.



Mineral Analysis

Mineral analyses were conducted at the Chemistry Advanced Research Centre, Sheda Science and Technology Complex, Abuja, following established protocols with modifications. Sample preparation involved dry ashing where 2g of each sample was weighed into porcelain crucibles and ashed in a muffle furnace (Carbolite Gero ELF) at 550°C for 5 hours. The ash was dissolved in 10ml of 6M HCl, filtered through Whatman No. 42 filter paper, and made up to 100ml with deionized water (AOAC, 2019). Mineral elements including iron, zinc, calcium, magnesium, potassium, and phosphorus were analyzed using Atomic Absorption Spectrophotometry (AAS; Thermo Scientific, iCE3000 AA02134104 v1.30) equipped with air-acetylene flame and specific hollow cathode lamps for each element. Phosphorus was determined spectrophotometrically using the vanadomolybdate method. Calibration curves were prepared using certified standard solutions (Sigma-Aldrich) for each mineral, with correlation coefficients (R^2) exceeding 0.999. All analyses were performed in triplicate, and results were expressed as mg/100kcal to facilitate comparison with RUSF standards.

Vitamin Analysis

Vitamin analyses were conducted at the Biochemistry Laboratory, Bayero University Kano, using validated methods. Fat-soluble vitamins (A, D, E, K) were extracted using saponification followed by hexane extraction and determined using High-Performance Liquid Chromatography (HPLC; Shimadzu LC-20AD) with UV detection (Achikanu et al., 2013). Water-soluble vitamins were analyzed using specific spectrophotometric methods: vitamin C was determined by the 2,6-dichlorophenolindophenol titration method; thiamine (B1) by the thiochrome method; riboflavin (B2) by fluorometric measurement; niacin (B3) by the Konig reaction; pyridoxine (B6) by HPLC with fluorescence detection; and folate (B9) by microbiological assay using *Lactobacillus casei* (AOAC, 2019). All analyses included appropriate quality controls, internal standards, and were performed in triplicate under amber light to prevent photodegradation.

Statistical Analysis

All analytical determinations were performed in triplicate, and results were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using SPSS Version 28.0 (IBM Corporation, Armonk, NY). Data normality was assessed using Shapiro-Wilk test. One-way ANOVA with LSD post-hoc test was used for multiple comparisons between formulations, and independent t-tests were used for paired

comparisons between FPP A and control. For sensory evaluation, non-parametric tests (Kruskal-Wallis with Dunn's post-hoc) were used where data violated normality assumptions. Significance was defined at $p < 0.05$ for all statistical tests.

Results and Discussion

Mineral Adequacy and Disparities in the Formulated Flour

The mineral analysis of the Formulated Food Products (table 1) (FFPs) reveals a promising and varied profile when benchmarked against the requirements of Ready-to-Use Supplementary Food (RUSF) and Growth and Recovery–High Macronutrient (GR-HM) standards.

In the current study, Iron in FFP A (11.38 mg/100kcal) and the Control (10.23 mg/100kcal) have the highest values, similar to that of GR-HM standards ($p < 0.05$). RUSFs are designed to deliver a high bioavailability of iron, often using chelated forms like ferrous fumarate or sodium iron EDTA, to reverse deficiency. FFPA met this standard for iron. This indicated that FFPA can be used to address Iron deficiency, which is a pervasive health burden in Nigeria, particularly affecting women and children (Osibogun et al., 2021).

In the results for zinc, only the Control (14.59 mg/100kcal) exceeds the standard (12.5 mg/100kcal). FFP A (10.09 mg/100kcal) is slightly below, and FFP B and C are significantly deficient. Zinc's role in linear growth and immune function makes this a limitation for products intended to support recovery from malnutrition (Lazzerini et al., 2020). The formulations demonstrate remarkable success in delivering other essential minerals, the Phosphorus, Potassium, and Magnesium. Phosphorus in FFP A (752.66 mg/100kcal) is exceptionally high, far exceeding both the RUSF (600 mg/100kcal) and the more demanding GR-HM (700 mg/100kcal) standards. Phosphorus is vital for energy metabolism (ATP) and is a key component of bone. This high level is a major strength, supporting the energy-dependent processes of growth (Bahwere, 2021). For Potassium, all products comfortably exceeded the RUSF standard (1150 mg/100kcal), which is crucial for maintaining electrolyte balance, nerve function, and cardiovascular health. For Magnesium, FFP A and B also showed strong magnesium content (~184-214 mg/100kcal), significantly higher than the other samples. Magnesium is a cofactor in over 300 enzymatic reactions and is often overlooked in fortification strategies (Lazzerini et al., 2020). Its adequate inclusion in these formulations is a positive finding. The calcium content showed high variability. FFP C (723.62 mg/100kcal) exceeded the RUSF standard (642 mg/100kcal), and FFP B was close, while FFP A and the Control were slightly below.

Vitamin Content in Formulated Food Products

The analysis of the vitamin content in Formulated Food Product (table 4.2) (FFP) A and the Control sample reveals a profile of significant strengths and specific, critical gaps when measured against the Ready-to-Use Supplementary Food (RUSF) standard. Vitamins are essential cofactors in metabolic pathways, and their adequate provision in therapeutic foods is non-negotiable for supporting immune function, energy metabolism, and overall recovery in malnourished individuals. In this study, Vitamin A content in FFP A (343.81 $\mu\text{g}/100\text{g}$) and the Control, while statistically similar, are significantly below the RUSF standard (425 $\mu\text{g}/100\text{g}$). Vitamin A deficiency is a major public health problem in Nigeria, leading to xerophthalmia, impaired immunity, and increased childhood mortality (Osibogun et al., 2021). Vitamin B2 (Riboflavin) and B3 (Niacin) contents while similar between

the samples, falls below the standard. These B-vitamins are integral to energy metabolism. Riboflavin deficiency can impair iron metabolism, exacerbating anemia, while niacin deficiency is associated with pellagra (Olagunju & Arigbede, 2022). The ascorbic acid content, though high and similar between samples (57-58 mg/100g), falls just below the RUSF standard (60 mg/100g). Vitamin C's role is twofold, it is a potent antioxidant and, more importantly, it significantly enhances the absorption of non-haem iron from plant-based foods (López et al., 2023).

B-Vitamins contents in the products performed excellently for Thiamine (B1), Pyridoxine (B6), and Folate (B9). The levels for B1 and B6 significantly exceed the RUSF standard, while folate meets it exactly. This is a major strength, as these vitamins are critical for neurological development, amino acid metabolism, and the prevention of neural tube defects and megaloblastic anaemia (Adeyemi et al., 2022).

Fat-Soluble Vitamins (D, E, K) in the formulation are particularly successful. The Vitamin D content meets the standard, which is crucial for calcium absorption and bone health anemia (Adeyemi et al., 2022). Vitamin E, a key antioxidant that protects the product's own unsaturated lipids from oxidation, meets the standard. Vitamin K, essential for blood coagulation, is very close to the standard with no statistical difference. This indicates a well-calculated and stable fat-soluble vitamin premix.

Conclusion

This comprehensive evaluation demonstrates that the plant-based RUSF cookie formulation possesses significant nutritional strengths, including B-vitamins and Minerals content;. The favourable minerals and vitamins content suggest that it can potentially be used in mitigating the deficiencies of these micronutrients. Additional research should investigate the impact of specific processing modifications on both nutritional properties to develop an optimal product that successfully bridges the gap between nutritional adequacy and consumer acceptability for effective management of moderate acute malnutrition in Nigeria.

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Table 1: Minerals Analysis of the FPPs, expected range for RUSF, expected range for GHM and Controls

Sample	Zinc (mg/100 kcal)	Magnesium (mg/100 kcal)	Phosphorus (mg/100 kcal)	Potassium (mg/100 kcal)	Calcium (mg/100 kcal)	Iron (mg/100 kcal)
FPP A	10.09±0.12 ^{ab}	213.71±0.68 ^a	752.66±1.11 ^a	1320.79±0.18 ^a	529.15±0.26 ^a	11.38±0.09 ^a
FPP B	8.92±0.04 ^b	184.36±0.40 ^a	306.82±0.02 ^b	1220.09±0.10 ^a	621.69±0.05 ^{ab}	8.61±0.02 ^b
FPP C	7.90±0.03 ^b	139.25±0.0 ^b	324.71±0.02 ^b	1141.97±0.05 ^a	723.62±0.04 ^b	4.53±0.06 ^c
Control	14.59±0.07 ^a	126.78±0.0 ^b	598.16±0.48 ^c	1198.73±0.3 ^a	533.91±0.03 ^a	10.23±0.11 ^{ab}
expected range for RUSF	12.50±0.0 ^a	187.50±0.0 ^a	600.00±0.0 ^c	1150.00±0.0 ^a	642.00±0.0 ^{ab}	12.00±0.0 ^a
expected range for GHM	12.50±0.0 ^a	310.00±0.0 ^c	700.00±0.0 ^{ac}	3500.00±0.0 ^b	1200.00±0.0 ^c	12.50±0.0 ^a
F-value	10.098	108.150	176.173	1276.672	150.616	20.533
P<value	<0.05	<0.001	<0.0001	<0.0000011	<0.0000001	<0.05

Values are mean±standard error of the mean of the Minerals contents of the FPP samples analysed. Values with different superscript on the same column are significantly different at $p<0.05$ at 95 % confidence interval. Expected range for RUSF (Goal Global 2023).

Table 4.2: Vitamins Analysis of the FPPs, expected range for RUSF, expected range for GHM and Controls

Sample	Vitamin A (µg/100g)	Vitamin B1 (mg/100g)	Vitamin B2 (mg/100g)	Vitamin B3 (mg/100g)	Vitamin B6 (mg/100g)	Vitamin B9 (mg/100g)	Vitamin C (mg/100g)	Vitamin D (µg/100g)	Vitamin E (mg/100g)	Vitamin K (µg/100g)
FPP A	343.81±0.4 8 ^a	1.45±0.02 ^a	1.52±0.0 2 ^a	10.57±0.08 a	2.53±0.02 ^a	343.41±1.22 ^a	57.83±0.22 a	11.73±0. 01 ^a	16.11±0. 05 ^a	9.78±0.08 ^a
FPP B	241.50±0.2 9 ^b	0.45±0.02 ^b	1.36±0.0 4 ^a	4.82±0.04 ^b	0.44±0.02 ^b	191.77±0.29 ^b	37.80±- .20 ^b	4.75±01. 2 ^b	6.66±0.2 9 ^b	3.88±0.04 ^b
FPP C	227.33±0.5 5 ^b	0.39±0.02 ^b	1.05±0.0 2 ^b	3.04±0.04 ^b	0.55±0.01 ^b	168.89±0.18 ^b	29.31±0.52 b	4.28±0.0 1 ^b	4.32±- 0.04 ^b	3.05±0.02 ^b
Control	381.59±0.4 6 ^{ac}	1.42±0.01 ^a	1.90±0.0 2 ^c	9.22±0.02 ^a	2.45±-.01 ^a	338.61±0.43a	58.73±0.08 a	11.68±0. 01 ^a	15.48±0. 03 ^a	9.23±0.02 ^a
expected range for RUSF	275-575	1	2.1	13	1.8	330	60.00±0.0 ^a	10.00±0. 0 ^a	15.00±0. 0 ^a	10.00±0.0 ^a
expected range for GHM	800-1000	1.2	1.2	15	1.3	400	100.00±0.0 c	12.50±0. 0 ^a	9.00±0.0 c	60.00±0.0 ^c
F-value	126.377	88.249	19.014	175.387	354.239	139.581	44.674	47.945	63.110	19.180

Values are mean±standard error of the mean of the Vitamins contents of the FPP samples analysed. Values with different superscript on the same column are significantly different at $p<0.05$ at 95 % confidence interval. Expected range for RUSF (Goal Global 2023).