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Optimization of a Multi-Micronutrient Powder Formulation Incorporating *Moringa oleifera*: A Sustainable Approach to Nutritional Intervention

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ABSTRACT

Micronutrients are essential nutrients that play a crucial role in supporting human health, survival, and overall bodily functions. Micronutrient deficiencies remain a critical public health concern, particularly in developing regions where malnutrition is prevalent. This study aimed to formulate and optimize a micronutrient powder blend using locally sourced ingredients which are Moringa, Spinach, Carrot, and Eggshell to address micronutrient deficiencies and improve dietary intake. Three formulations (F1, F2, and F3) were developed to evaluate their nutritional composition, functional properties, storage stability, and sensory acceptability. The study employed an experimental design, including proximate analysis, micronutrient profiling, assessment of antinutritional factors, and sensory evaluation. The results showed that the three formulations contained varying levels of essential micronutrients, with F3 exhibiting the highest calcium (369.43 mg/100 g), iron (393.97 mg/100 g), and vitamin A (126.40 µg/g) content. Functional properties such as bulk density (0.48–0.50 g/cm³), water adsorption capacity (1.26–1.30 g/g), and swelling capacity (1.32–1.35 g/g) were within acceptable ranges. Storage stability analysis indicated minimal nutrient degradation over time, with peroxide values ranging from 8.30 to 8.73 meq/kg. Sensory evaluation revealed that F1 had the highest overall acceptability, particularly in color (8.33), taste (8.33), and texture (7.67). These findings suggest that the developed micronutrient powder formulations could serve as viable dietary supplements to combat micronutrient deficiencies. Further studies are recommended to assess bioavailability, large-scale production feasibility, and potential health impacts.

Keywords: *Micronutrient deficiency, Moringa oleifera, Carrot, Spinach, Egg Shell, nutritional formulation, Minerals, Vitamins, Dietary Supplementation, Nutritional Quality*

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INTRODUCTION

Micronutrient deficiency also known as hidden hunger is a form of undernutrition that occurs when intake or absorption of micronutrients such as iron, zinc, iodine and vitamin A is too low to maintain good health and development (Biesalski, 2013). Contributing factors take account of poor maternal and child dietary patterns, limited access to nutritious food, increased micronutrients demand by body during pregnancy, lactation period as well as childhood stage below five years of age (von Grebmer *et al.,* 2014; Ojiewo *et al.,* 2015).

Globally, hidden hunger afflicts more than two billion people (Hoddinott, Rosegrant, and Torero, 2012).

Iron, zinc, iodine and vitamin A are the big four micronutrients of public health concern. Zinc deficiency is indicated by a weak immune system and stunting, affecting 1.2 billion people, of which 165 million are stunted children (Bhutta *et al.,* 2013). Iron deficiency disorder affects 1.6 billion people with nutritional anaemia, reduced physical activities, impaired cognitive development and risks of maternal death, low birth weight and premature birth (de Benoist *et al.,* 2018). Vitamin A deficiency is implicated by night blindness (xerophthalmia), irreversible blindness, poor epithelial health and weak immune system as well as risk of death in both pregnant women and preschool-aged

children (von Grebmer *et al.,* 2014). Global reports indicate that 19 million pregnant women and 190 million preschool children are vitamin A deficient (World Health Organization, 2009). Developing countries are at higher risks of hidden hunger as they are moving from traditional diets with minimally processed food to highly processed foods that are high in calories but poor in micronutrients. Moreover, these diets rely much on single staple foods that are energy dense, contributing to high levels of micronutrient deficiency in women at reproductive age and children below 5 years (Pauw and Thurlow, 2011).

Increasing dietary diversification is one of the effective and sustainable interventions to prevent hidden hunger. At the same time, food fortifications with iron, iodine and vitamin A during processing have also proved to reduce micronutrient deficiencies (Bouis and Saltzman, 2017). However, food fortification is affected by target micronutrient stability, bioavailability, not reaching the target population, overconsumption of micronutrients and monitoring of an individual taking fortified food (Dwyer *et al.,* 2015).

Micronutrient deficiency is increasing due to an ever-increasing global population. Iron and Zn deficiency can cause serious health problems such as impaired cognitive and mental development, decreased growth, anemia, immune system impairment, and increased death rate (Black, 2003). Micronutrient malnutrition not only increases disease burden, but also deteriorates social welfare and economic productivity on a global scale (Stein, 2010). Currently, micronutrient malnutrition evolving from Zn and Fe deficiencies is affecting 3 billion people worldwide with serious health effects. The major reason for increased prevalence of Zn and Fe deficiencies has been traced back to staple food-based diets that are low in nutrition, for example, Zn and Fe.

Zinc deficiency contributes to the death of 800,000 children globally per year (Hagan *et al.* 2010). Deficiency of copper, zinc, and manganese impairs cell functions and affects growth and development, immune system, and metabolism. Copper is the primary constituent of cuproenzymes, which are involved in energy production, iron metabolism, and neurotransmitter synthesis metabolism (Mejía-Rodríguez *et al.* 2013). Zinc is also important in neurologic function (Yamada *et al.* 2014).

Moringa oleifera, a green leafy vegetables is a rich source of vitamins and dietary minerals such as iron, calcium, zinc, and manganese (Singh *et al.,* 2001; Seshadri and Nambiar 2003; Sheela *et al.* 2004; Fahey 2005; Odhav *et al.* 2007; Raju *et al.* 2007; Oduro *et al.* 2008). These micronutrients are essential to the human body because they perform important biochemical roles needed for general well-being. Iron (Fe) deficiency, for example, leads to iron deficiency disorders including anemia which affects about a third of the world's population (Yang *et al.,* 2023); vitamin A deficiency is associated with vitamin A deficiency disorders including night blindness, increased risk of resistance to severe infection and impaired embryonic development and spermatogenesis in males (Clagett-Dame and Knutson 2011; World Health Organization (WHO, 1995).

The nutritional profiles of *Moringa oleifera*, spinach, carrot, and eggshells present a compelling case for their optimization in micronutrient powder development. *Moringa oleifera* stands out with its exceptional nutrient density; it is rich in vitamins A, C, and E, as well as essential amino acids and minerals such as calcium and potassium, making it a potent candidate for combating malnutrition. Spinach, similarly, contributes a wealth of vitamins, particularly vitamin K and folate, which are critical for blood health and cellular function. Carrots offer a high concentration of beta-carotene, an important precursor to vitamin A, enhancing immune function and vision. Lastly, eggshells provide a natural source of calcium carbonate, crucial for bone health and various metabolic processes. Combined, these ingredients not only enhance the nutritional quality of the powders but also support diverse dietary needs, promoting overall health and wellness.

The comparative analysis of micronutrient content in selected ingredients such as *Moringa oleifera*, spinach, carrots, and eggshells reveal significant differences in their nutritional profiles, which is crucial for the development of optimized micronutrient powders. *Moringa oleifera* stands out due to its exceptionally high levels of vitamins A, C, and E, which serve as potent antioxidants, as detailed in recent studies highlighting the role of antioxidant molecules in combating oxidative stress and chronic diseases (Frano *et al.,* 2014; Welch and Graham, 1999). Spinach contributes valuable iron and folate, which are essential for blood health and cellular functionality, while carrots are known for their beta-carotene content, vital for vision and immune function. Eggshells, primarily composed of calcium carbonate, can further enhance the mineral profile of these powders, providing a bioavailable source of calcium critical for bone health.

Despite advancements in nutritional science, micronutrient deficiencies remain a significant public health concern, particularly among vulnerable populations such as women of reproductive age. These deficiencies can lead to serious health issues, including anemia, impaired immune function, and complications during pregnancy and childbirth (WHO, 2020). In Nigeria, micronutrient malnutrition is prevalent, with a substantial portion of the population not meeting their daily recommended intake of essential vitamins and minerals (Maziya-Dixon *et al.,* 2004). A National Food Consumption and Micronutrient Survey conducted in 2021 revealed that a significant portion of the Nigerian population, including those in Kebbi State, fails to meet the dietary requirements for essential nutrients like iron, vitamin A, and zinc (FGN and International Institute of Tropical Agriculture, 2021). The survey highlighted the critical need for interventions to improve the nutritional status of women and children in these regions. Therefore, this study aimed to formulate and optimize a micronutrient powder blend using *Moringa oleifera* and other natural ingredients to populations affected with micronutrient deficiencies.

MATERIALS AND METHODS

All chemicals and reagents used were of analytical grade and were purchased from Sigma Aldrich.

Procurement of experimental materials

Experimental materials for the micronutrient powder formulation were selected based on local availability, cultural acceptability, nutritional composition, and affordability. Moringa *(Moringa oleifera),* Spinach *(Spinacia oleracea),* Carrot *(Daucus carota)* was obtained from Birnin Kebbi Central market while egg shell was collected from household and restaurant waste in Birnin Kebbi, Kebbi State.

Sample preparation

Moringa oleifera, *Spinacia oleracea* and *Daucus carota* was sorted and cleaned using tap water to remove dust, debris and any foreign matters. The vegetables was blanched in boiling water at a temperature of 96.5 °C as described by Gamboa-Santos *et al.* (2013) and Patras, Tiwari, and Brunton (2011). Blanching was performed in order to inactivate enzymes to prevent enzymatic browning and micronutrients oxidation, sterilizing vegetables, structural softening to facilitate moisture removal during drying and evaporating herb-like flavours (Tanongkankit **et al.,** 2010). After blanching, the samples were loaded on the solar drier trays by spreading them to make a thin layer for effective drying during the morning hours at 0900 h. Drying was conducted from 0900 to 1700 h in the direct cabinet solar drier. Duration of drying was about 1-2 days. Constant moisture content was used to decide end of drying. Unloading of the dried vegetables from the drier was done in the afternoon when relative humidity was low to avoid moisture pickup. Dried vegetables was packed in polyethylene bags and stored at room temperature in a dark dry place for further analysis and novel product formulation.

The unboiled chicken eggshells were collected from household and restaurant in Birnin Kebbi, Kebbi State, Nigeria. The eggshells were washed with distilled water, without peeling off the membranes. It was then sun-dried to constant weight, ground to a powder using a grinding machine and sieved to a fine powder by using a 0.5 micrometer sized sieve. The sieved eggshell was oven-dried at 38 °C until constant weight was obtained. The prepared eggshell was then stored in clean, airtight containers to prevent moisture absorption and contamination.

Formulation of micronutrient powder

The ingredients were mixed in different formulation ratios of F1 (4:3:2:1), F2 (5:2:2:1) and F3 (3:4:2:1) for *Moringa oleifera, Daucus carota, Spinacia oleracea* and Egg shell respectively on dry weight. The powder was packed in a container and stored in a dark dry place at room temperature before further analysis (Table 1).

Table 1: Composition (g) of the micronutrient powder per 100g dry weight.

INGREDIENTS	F1	F2	F3
Moringa oleifera	40	50	30
Daucus carota	30	20	40
Spinacia oleracea	20	20	20
Egg shell	10	10	10

Proximate analysis

Determination of moisture content

Moisture content was determined using the oven-drying method (AOAC, 2019). A known weight of the sample was placed in a pre-weighed crucible and dried in a hot air oven at 105°C for 24 hours until a constant weight was obtained. The crucible was then cooled in a desiccator and reweighed. Moisture content was calculated as:

$$Moisture\ Content\ (\%) = \left(\frac{Initial\ Weight}{Residue\ Weight}\right) X\ 100$$

Determination of ash content

Ash content was determined by incinerating the sample in a **muffle furnace at 550°C for 4 –6 hours** (AOAC, 2019). A pre-weighed crucible containing the sample was placed in the furnace until all organic matter was burnt off, leaving a white or grayish residue. The crucible was cooled in a desiccator and reweighed. The ash content was calculated as:

$$Ash\ Content\ (\%) = \left(\frac{Initial\ Weight}{Residue\ Weight}\right) X\ 100$$

Determination of crude fiber

Crude fiber was determined using the acid and alkali digestion method (AOAC, 2019). The sample was defatted using petroleum ether and then digested sequentially with 1.25% sulfuric acid and 1.25% sodium hydroxide solution under controlled conditions. The insoluble residue was filtered, dried, incinerated in a muffle furnace at 550°C, and weighed. The crude fiber content was calculated as:

$$Crude\ Fiber\ (\%) = \left(\frac{Loss\ in\ Weight\ on\ Ignition}{Sample\ Weight}\right) X\ 100$$

Determination of crude protein

The crude protein content was determined using the Kjeldahl method (AOAC, 2019). The sample was digested

with concentrated sulfuric acid (H_2SO_4) and a catalyst in a digestion flask until a clear solution was obtained. The digest was neutralized with sodium hydroxide (NaOH) and distilled into boric acid solution, followed by titration with standard hydrochloric acid (HCl). The total nitrogen content was determined, and protein content was calculated using a conversion factor of 6.25:

$$Crude\ Protein\ (\%) = Total\ Nitrogen\ X\ 6.25$$

Determination of Lipid Content (Soxhlet Extraction Method, AOAC 963.15)

Lipid content was determined using the Soxhlet extraction method (AOAC, 2019). A pre-weighed dry sample was placed in a thimble and extracted with petroleum ether as a solvent for 4–6 hours. After extraction, the solvent was evaporated, and the lipid residue was dried and weighed. The lipid content was calculated as:

$$Lipid\ Content\ (\%) = \frac{Weight\ of\ Extracted\ Fat}{Sample\ Weight} X\ 100$$

Determination of Carbohydrate Content (By Difference)

Carbohydrate content was determined by subtracting the sum of moisture, ash, crude fiber, protein, and lipid contents from 100% (FAO, 2003).

$$Carbohydrate\ (\%) = 100 - (Moisture + Ash + Fibre + Protein + Lipid)$$

Determination of minerals

The mineral content was determined using the Atomic Absorption Spectrophotometer (AAS) (Buck Scientific Atomic Absorption Emission Spectrophotometer model 205, manufactured by Norwalk, Connecticut, USA) using standard wavelengths. Two grams of the sample was ashed following standard AOAC (2010) methods. The ashed sample was digested with 2.5 ml of 0.03 N hydrochloric acid (HCl). The digest was boiled for 5 min, allowed to cool to room temperature and transferred to a 50 ml volumetric flask and made up to the mark with distilled water. The resulting digest was filtered with ashless Whatman No. 42 filter paper. The filtrate from each sample was analyzed for mineral content (iron, zinc, calcium, magnesium, potassium) using an AAS. The real values of the minerals were extrapolated from the respective standard curves and values obtained were adjusted for HCl-extractability for the respective ions. The absorbance for calcium, magnesium, iron, and potassium was read at 422.7, 285.2, 372.0, 766.5 nm, respectively.

Determination of Vitamin content

Determination of Vitamin A (Beta-Carotene and Retinol)

The vitamin A content was determined following the AOAC 2010 method 970.64 (AOAC, 2010). One gram (1 g) of the sample was weighed and extracted with 20 mL of cold acetone in a dark environment to prevent degradation. The mixture was filtered, and 20 mL of petroleum ether was added to the filtrate to partition the carotenoids. The lower aqueous phase was discarded, and the upper organic layer was washed with distilled water to remove acetone. The extract was evaporated under nitrogen gas to dryness, and the residue was dissolved in 1 mL of hexane. The absorbance was measured at 450 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Beta-carotene concentration was calculated using the following equation:

$$Concentration\ (mg) = \frac{Abs\ X\ Volume\ of\ cuvette\ X\ D_f}{E}$$

Where:

- Abs = Absorbance
- Df = Dilution factor
- E = Extinction coefficient (2592 for beta-carotene in hexane)

Determination of Vitamin C (Ascorbic Acid)

The vitamin C content was determined using the 2,6-Dichlorophenol-Indophenol (DCPIP) titration method (AOAC 2010, 967.21). One gram (1 g) of the sample was homogenized with 50 mL of 3% metaphosphoric acid solution to stabilize ascorbic acid. The mixture was centrifuged at 5000 rpm for 10 minutes to obtain a clear supernatant. The extract was titrated with freshly prepared 0.01% DCPIP solution until a pink color persisted for 30 seconds. The ascorbic acid content was calculated using the equation:

$$Vitamin\ C\ (mg) = \frac{V_1\ X\ C_1}{V_2}$$

Where:

- V_1 = Volume of DCPIP used (mL)
- C_1 = Concentration of standard ascorbic acid solution
- V_2 = Volume of the sample extract

Determination of Vitamin E (Tocopherols)

The vitamin E content was analyzed using HPLC with UV detection according to AOAC 2010, Method 992.03. One gram (1 g) of the sample was extracted with 10 mL of hexane and vortexed for 5 minutes. The mixture was centrifuged at 3000 rpm for 10 minutes, and the supernatant was collected. The extract was evaporated to

dryness under nitrogen gas and reconstituted in 1 mL of mobile phase (methanol:acetonitrile, 50:50 v/v). A C18 column (250 mm × 4.6 mm, 5 µm) was used with a mobile phase flow rate of 1.0 mL/min. The detection wavelength was 292 nm (specific for α-tocopherol). Tocopherol concentration was calculated from a calibration curve using standard α-tocopherol.

Determination of Vitamin K (Phylloquinone)

Vitamin K (phylloquinone) was analyzed using HPLC with fluorescence detection as per AOAC 2010, Method 999.15. One gram (1 g) of the sample was extracted with 15 mL of hexane-ethanol (80:20 v/v) and vortexed for 5 minutes. The mixture was centrifuged at 4000 rpm for 10 minutes, and the supernatant was collected. The extract was evaporated under nitrogen gas and reconstituted in 1 mL of mobile phase (methanol: water, 75:25 v/v). The separation was carried out on a C18 column (150 mm × 4.6 mm, 5 µm). The mobile phase flow rate was 1.2 mL/min, and fluorescence detection was performed at excitation 244 nm and emission 430 nm. Vitamin K concentration was determined using a calibration curve of standard phylloquinone.

Determination of Vitamin B9 (Folate)

1 g sample was weighed and placed into a centrifuge tube. Phosphate buffer (pH 6.0, 100 mM) containing ascorbic acid (1%) and dithiothreitol (DTT) (1%) was added to prevent oxidation. The mixture was vortexed and incubated at 37°C for 30 minutes to facilitate extraction. A conjugate enzyme was added to hydrolyze polyglutamate folates into monoglutamate forms. The solution was incubated at 37°C for 2 hours to allow for enzymatic action. The reaction was stopped by heating at 100°C for 3 minutes. The sample was then centrifuged at 10,000 rpm for 10 minutes to separate the supernatant. The supernatant was filtered through a 0.22 µm membrane filter to remove impurities and the filtered extract was collected and stored for HPLC analysis.

HPLC Conditions

- Column: C18 reversed-phase column (250 mm × 4.6 mm, 5 µm) was used for chromatographic separation.
- Mobile Phase: A solution of 50 mM phosphate buffer (pH 2.3) with 10% acetonitrile was used.
- Flow Rate: The mobile phase was pumped at a flow rate of 1.0 mL/min.
- Injection Volume: A 20 µL aliquot of the sample was injected into the HPLC system.
- Run Time: The total runtime for each analysis was approximately 25 minutes.

Post-Column Derivatization (Iodine Oxidation)

- As the eluted folate compounds passed through the column, they were subjected to post-column oxidation with iodine, which converted them into fluorescent pterin derivatives.
- The oxidation reaction was carried out in a heated reaction coil at **95°C**.

Fluorescence detection and quantification

- The derivatized folates were detected using fluorescence detection at an excitation wavelength of 290 nm and an emission wavelength of 360 nm.
- The peak areas of the folate compounds were recorded and compared to a standard calibration curve generated using folic acid standards of known concentrations.

Calculation of folate content

The folate concentration in the sample was calculated using the equation:

$$Folate\ Concentration\left(\mu\frac{g}{g}\right) = \frac{Peak\ Area\ of\ Sample\ X\ Dilution\ Factor}{Standard\ Curve\ Slope}$$

Determination of some selected functional properties

Determination of Bulk Density (BD)

The bulk density of the formulation was determined according to the method of Okaka (2005). A previously weighed measuring cylinder was filled to the 10 ml mark with the sample. The bottom of the cylinder was tapped gently but repeatedly on a laboratory bench until there was no further reduction of the sample level. The cylinder with the sample was weighed. The bulk density was calculated as:

$$BD\ (gcm^{-3}) = \frac{W_2 - W_1}{V}$$

Where
BD = bulk density (g/cm^3),
W_1 = weight of the empty cylinder (g),
W_2 = weight of cylinder + sample (g),
V = volume of cylinder occupied by the sample (cm^3).

Determination of water absorption capacity (WAC)

Water absorption capacity was determined using the method of Kanu *et al.* (2009). One gram of the sample was introduced into a weighed centrifuge tube with 10 ml of distilled water and mixed thoroughly. The mixture was allowed to stand for one hour before being centrifuged at 350 rpm for 30 min. The excess water (unabsorbed) was

decanted. The weight of water absorbed was determined by difference. The water absorption capacity was calculated using:

$$WAC = \frac{Volume\ of\ water\ used - Volume\ of\ free\ water}{Weight\ of\ the\ Sample} X100$$

Determination of swelling capacity (SWC)

This was determined using the method described by Jongaroontaprangsee *et al.,* (2007). Ten grams of the sample was measured into a 300 ml measuring cylinder. Then 150 ml of distilled water was added and allowed to stand for four hours. The final volume after swelling was recorded and percentage swelling capacity was calculated as:

$$Swelling\ capacity(\%) = \frac{Final\ Volume - Initial\ Volume}{Initial\ Volume} X\ 100$$

Determination of viscosity

The viscosity was determined using a torsson viscometer (Gallenkamp, England). Ten percent (10%) (w/v) of the formulation was heated for 10 min at 100 °C to form a gruel. The gruel was cooled to 28 °C before viscosity measurement was taken from the viscometer.

Determination of pH

The pH of the micronutrient powder was measured in a 10% (w/v) dispersion of the samples in distilled water and mixed thoroughly. A standard pH meter (Hanna meter model H196107) was used for pH determination. The pH electrode was dipped into the solution and after a few minutes of equilibration, the pH of the samples was taken.

Determination of storage stability of the formulation

Peroxide Value determination

The peroxide value was determined using the method described by Sani (2015). 2.0 g of the flour sample was weighed into a clean dry flask and 22 cm^3 of the mixture of 10 cm^3 of acetic acid and 12 cm3 of chloroform was added, then 0.5 cm3 of potassium iodide was also added. The flask was closed and allowed to stay with constant shaking for 1 minute. 30 cm3 of distilled water was then added and titrated against 0.1 M of sodium thiosulphate ($Na_2S_2O_3$) solution until an initial yellow colour disappeared and a faint blue colour appeared. The titration continued after the addition of 0.5 cm3 of starch indicator until there was a sudden disappearance of the blue colour, which signifies the end point. The peroxide value is often reported as the mL of 20 mM $Na_2S_2O_3$ per gram of sample. Thus, peroxide value was calculated using the equation below.

$$Peroxide\ value = \frac{(S-B)\times M\times 100}{W}$$

Where:
Peroxide value= mEq of peroxide per 100 g of sample
S=sample titre value (cm^3)
B=blank titre value (cm^3)
M= molarity of $Na_2S_2O_3$ (mEq/cm^3)
W=weight of flour

Free fatty acids

Free fatty acid was determined according to the method of Sani (2015). 2.0 g of the flour was transferred into a 250 cm^3 Erlenmeyer flask followed by the addition of 100 cm^3 of ethanol and 2 cm^3 of phenolphthalein indicator. After mixing the content properly, it was titrated against 0.04 M NaOH. The shaking continued until a slight pink colour was observed, which was steady for about 30 seconds and signified the end point. The % of free fatty acids were calculated using the below equation.

$$\%FFA = \frac{V\times M\times 28.2}{W}$$

Where: % FFA = percentage of free fatty acids, V= average volume of NaOH used (cm^3), M = molarity of NaOH, W = weight of the flour sample.

Determination of moisture contents

Oven Drying Method, the most common and traditional method was used to determine the moisture content during storage. An empty crucible was weighed and recorded. Approximately 5 grams of the powdered formulation was weighed and recorded as the combined weight (dish + sample). The dish with the sample was placed in a preheated oven set at 105°C for about 2-4 hours or until a constant weight is achieved. The dish was removed from the oven, cooled in a desiccator, and weighed. The moisture content was calculated using the following formula:

$$Moisture\ Content\% = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} X\ 100\%$$

Sensory evaluation

The nine-point Hedonic scale was used to evaluate the sensory parameters which were colour, appearance, texture, taste, consistency, flavor and overall acceptability of the powder. Where 9 = like extremely, 8 = moderately like, 1 = extremely dislike. The evaluation was done by 20 semi trained panelists through random selection of students from the Department of Biochemistry and Molecular Biology, Federal University Birnin Kebbi. The formulated micronutrient powder was coded and presented in coded plastic plates to the panelists

randomly. The panelists were instructed not to make comments during evaluation to avoid influencing other panelists. They also make comment freely on sample based on the questionnaires given to them.

Determination of antinutritional factor

Determination of phytate

Phytic acid was determined as described by Wheeler and Ferrel (1971). Phytic acid was extracted from 3g of sample with 3% trichloroacetic acid by shaking at room temperature followed by high-speed centrifugation. Then the phytic acid was estimated by multiplying the amount of phytate-phosphorus by the factor 3.55.

Determination of oxalate

Oxalate content was determined according to AOAC (2005). 1g of sample was weighed into 100cm^3 conical flask. Then, 75cm^3 of 3 mol/l H_2SO_4 was added. The solution was stirred intermittently for 1hour on magnetic stirrer and then filtered using Whatman No. 1 filter paper. 25cm^3 of the sample filtrate was collected and titrated against hot (80-90˚C) 0.1 N $KMnO_4$ to point where a faint pink color appear that persist for 30 seconds. Oxalate was calculated using 1cm^3 0.1 permanganate = 0.006303g oxalate.

Determination of tannin

Tannin was determined using Folin-Denis spectrophotometric method. 1.0 g of the formulation was dispersed in 10 mL distilled water and agitated. This was allowed to stand for 30 mins at room temperature while continuously stirring every 5mins. After 30 mins, it was centrifuged and the extract was obtained. 2.5 mL of the extract was dispersed into a 50 mL volumetric flask. Similarly, 2.5 mL of standard tannic acid was dispersed into a separate 50ml flask. A 1.0 mL Folin-Denis reagent was measured into each flask and was followed by the addition of 2.5 mL of saturated Na_2CO_3 solution. The mixture was diluted and made up to the 50 mL mark of the flask and was incubated for 90 mins at room temperature. The absorbance was measured at 250 nm in a UV spectrophotometer; readings was taken with the blank sample at zero (AOAC, 2005).

The tannin content was given as follows;

$$\%\,Tannin = \frac{An}{As}\ X\ C\ X\ \frac{100}{w}\ X\ \frac{VF}{VA}$$

An= absorbance of the test sample
As = absorbance of standard solution
C = concentration of standard solution
W = weight of sample used
VF = total volume of extract
VA = volume of extract analyzed

Determination of nitrate

High-performance liquid chromatography (HPLC) was employed for precise nitrate quantification. Sample extraction was performed using an aqueous solution, followed by filtration. The extract was injected into an HPLC system equipped with a UV detector set at 210 nm**.** Nitrate ions were separated using a reversed-phase column and quantified based on peak area and retention time. Standard solutions were prepared to generate a calibration curve for accurate analysis (AOAC, 2005).

Determination of cyanide

The determination of cyanide in the formulation was conducted following the official method outlined by the Association of Official Analytical Chemists (AOAC, 2005). The sample was homogenized, and an appropriate amount was weighed accurately. It was then transferred into a distillation flask containing sodium hydroxide solution to trap any released hydrogen cyanide. The distillation apparatus was set up, and the sample was subjected to acid hydrolysis. This process facilitated the conversion of bound cyanide into free hydrogen cyanide (HCN), which was then volatilized and carried over into an alkaline absorbing solution. The distillation was continued until complete cyanide recovery was ensured. The collected distillate was treated with chloramine-T reagent to oxidize cyanide to cyanogen chloride. Pyridine-barbituric acid reagent was then added to react with the cyanogen chloride, forming a stable reddish-purple complex. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 580 nm. A calibration curve was prepared using standard potassium cyanide solutions. The absorbance values of the samples were compared against the calibration curve to determine the cyanide concentration. The results were expressed in mg/dl. The cyanide concentration was calculated using the standard calibration curve.

Statistical analysis

Data were reported as mean and standard deviation of triplicate determination. Data were subjected to one-factor analysis of variance (ANOVA) to test for significance variation (5%) of each micronutrient among formulations. Regression analysis was performed to find which formulation is associated more with micronutrients. Likewise, t-test was performed to compare for significance difference between micronutrients in the formulated novel product and *Moringa oleifera*. Values were analyzed using the SPPS statistical package.

Table 2: Vitamins Content in the Formulated Powder**.**

Nutrients	F1	F2	F3	*M. oleifera*
Vitamin A	139.60±1.31^{c}	132.67±2.66^{b}	126.40±0.96^{a}	113.00
Vitamin E	91.57±1.14^{b}	86.47±1.14^{a}	84.23±1.27^{a}	74.00
Vitamin K	194.77±1.43^{b}	191.57±1.30^{a}	189.57±0.78^{a}	152.00
Folic acid	61.77±1.12^{b}	60.03±0.75^{ab}	58.77±0.61^{a}	37.00
Ascorbic acid	3343.47±7.27^{a}	3513.40±10.60^{b}	3677.83±2.12^{c}	2200.00

Data are presented as mean ± SD for three replicate experiments. Means with different superscript letters within the same row are significantly different (P< 0.05; One-Way ANOVA with Tukey's post hoc test). A significant difference between the two groups of sample and standard determined using an independent T-test at P < 0.05.

RESULTS AND DISCUSSION

The micronutrient analysis of the formulation reveals measurable amounts of key vitamins that are essential for women of reproductive age. Although the reported values are per gram of the powder, they indicate that with an appropriate serving size, this powder can contribute to the daily nutritional needs. Vitamin A is crucial for maintaining healthy vision, immune function, and cellular differentiation. In women of reproductive age, adequate vitamin A is important not only for general health but also for supporting fetal development once pregnancy is achieved. The vitamin composition of the formulated micronutrient powders (F1, F2, and F3) was analyzed and compared with the reported values for *Moringa oleifera* leaves. Notable variations were observed among the formulations, with differences in vitamin concentrations likely influenced by the proportions of ingredients used.

From (Table 2) Vitamin A content was highest in F1 (139.6 ± 2.53 µg/g), followed by F2 (132.7 ± 2.53 µg/g) and F3 (126.4 ± 2.53 µg/g). These values exceeded the reported vitamin A concentration in *Moringa oleifera* (113.00 µg/g) (FAO, USDA). The higher levels observed in the formulations suggest a potential enhancement in vitamin A content due to the contribution of other vitamin-rich ingredients, which may improve the prevention of vitamin A deficiency-related disorders, such as night blindness and impaired immune function (Olson, 2020). This is not just a supplemental contribution, it is designed to provide a complete dietary requirement of vitamin A (Mayo Clinic, 2021).

As a powerful fat-soluble antioxidant, vitamin E helps protect cells from oxidative stress, a factor implicated in reproductive disorders such as polycystic ovary syndrome (PCOS). The RDA for vitamin E is about 15 mg/day (15,000 µg/day) for women of reproductive age (Traber and Atkinson, 2007). From (Table 2), Vitamin E levels followed a similar trend, with F1 (91.6 ± 3.62 µg/g) containing the highest concentration, followed by F2 (86.5 ± 3.62 µg/g) and F3 (84.2 ± 3.62 µg/g). These values were slightly higher than the 74.00 µg/g reported for *Moringa oleifera* (FAO). Vitamin E is a crucial antioxidant that protects cell membranes from oxidative stress, reducing the risk of chronic diseases such as cardiovascular disorders (Traber and Atkinson, 2007). The elevated vitamin E content in the formulations indicates their potential contribution to antioxidant defense mechanisms. Vitamin K is essential for blood clotting and bone health, and it plays a role in the regulation of calcium metabolism. For women of reproductive age, the RDA is approximately 90 µg/day (Shea and Booth, 2019). According to the results in Table 2, Vitamin K was found to be highest in F1 (194.8 ± 3.81 µg/g), followed by F2 (191.6 ± 3.81 µg/g) and F3 (189.6 ± 3.81 µg/g), all of which exceeded the reported *Moringa oleifera* content (152.00 µg/g) (USDA). The enhanced levels may be attributed to the presence of additional vitamin K-rich components in the formulations. Vitamin K is essential for blood coagulation and bone metabolism, and its increased presence in the formulations could be beneficial in reducing the risk of hemorrhagic conditions and osteoporosis (Shearer and Newman, 2019). It is also beneficial particularly during pregnancy and lactation, when calcium balance and bone health are critical (Vermeer and Theuwissen, 2020).

Folic acid (vitamin B9) is vital for DNA synthesis and red blood cell production, and it is especially important in preventing neural tube defects in the developing fetus. The recommended intake for women of reproductive age is about 400 µg/day, with even higher needs during pregnancy (National Institutes of Health, 2021). Folic acid content was higher in the formulations compared to *Moringa oleifera*. From (Table 2), the highest concentration was recorded in F1 (61.8 ± 3.24 µg/g), followed by F2 (60.0 ± 3.24 µg/g) and F3 (58.8 ± 3.24 µg/g), whereas *Moringa oleifera* leaves contained 37.00 µg/g (FAO). This means that the formulations provide a significant source of folic acid, which is essential for DNA synthesis and red blood cell formation, playing a crucial role in preventing neural tube defects during pregnancy (Bailey *et al.,* 2015).

Vitamin C is an essential water-soluble antioxidant that aids in collagen synthesis, enhances iron absorption, and supports immune function. The RDA for women of reproductive age is around 75 mg/day (75,000 µg/day) (National Institutes of Health, 2021). Ascorbic acid (vitamin C) content was significantly higher in all formulations compared to *Moringa oleifera* (2200.00 µg/g) (USDA). From (Table 2), the highest concentration was observed in F3 (3677.8 ± 10.26 µg/g), followed by F2 (3513.4 ± 10.26 µg/g) and F1 (3343.5 ± 10.26 µg/g). The increase in ascorbic acid levels suggests an enhanced antioxidant

Table 3: Minerals content in the formulated powder.

Nutrients	F1	F2	F3	*M. oleifera*
Calcium	354.27±3.59[a]	362.87±2.80[b]	369.43±1.33[b]	4.40
Iron	365.13±1.81[a]	382.40±1.81[b]	393.97±2.60[c]	0.70
Potassium	574.50±2.12[a]	574.90±1.40[a]	573.73±1.11[a]	1.32
Magnesium	141.37±1.02[a]	140.87±1.15[a]	140.10±1.11[a]	0.36
Zinc	20.17±0.23[a]	20.00±0.26[a]	19.83±0.31[a]	0.60

Data are presented as mean ± SD for three replicate experiments. Means with different superscript letters within the same row are significantly different (P< 0.05; One-Way ANOVA with Tukey's post hoc test). A significant difference between the two groups of sample and standard determined using an independent T-test at P < 0.05.

potential of the formulations, which could improve immune function, promote iron absorption, and reduce the risk of scurvy (Carr and Maggini, 2017). The contribution will be modest but when it is consumed in large quantity it will meets its daily requirement. Its role is complementary, ensuring a balanced antioxidant intake that supports overall cellular health (Carr and Maggini, 2017).

For women in their reproductive years, ensuring an adequate intake of these vitamins is critical for maintaining overall health, supporting fertility, and safeguarding fetal development. While the micronutrient powder provides measurable amounts of each vitamin, its efficacy depends on the serving size and the context of the overall diet.

The micronutrient composition of the formulated powder highlights its potential as a rich source of essential minerals. The mineral composition of the formulated micronutrient powders (F1, F2, and F3) was analyzed and compared to the reported values for *Moringa oleifera* leaves. Variations were observed among the formulations, with differences in mineral content likely influenced by the selection and proportion of ingredients used in the formulations.

Calcium is essential for bone health, nerve transmission, and muscle function. The Recommended Dietary Allowance (RDA) for calcium is 1000 mg/day for women of reproductive age and increases to 1300 mg/day during pregnancy and lactation (National Institutes of Health, 2021). Table 3 shows that Calcium content was highest in F3 (369.4 ± 21.38 mg/g), followed by F2 (362.9 ± 21.38 mg/g) and F1 (354.3 ± 21.38 mg/g), all of which were significantly higher than the reported *Moringa oleifera* calcium concentration of 4.40 mg/g (FAO). The elevated calcium levels in the formulations suggest an enhanced potential for supporting bone mineralization, muscle contraction, and nerve function, thereby reducing the risk of osteoporosis and other calcium deficiency-related disorders (Weaver, 2014). The high calcium content in the formulation, likely derived from eggshells, makes it a valuable dietary supplement to prevent osteoporosis and support fetal skeletal development (Dawson-Hughes *et al.,* 1990).

Iron is critical for oxygen transport, energy metabolism, and red blood cell formation. The RDA for iron is 18 mg/day for women of reproductive age and increases to 27 mg/day during pregnancy (WHO, 2020). As shown in (Table 3), the highest iron content was observed in formulation F3 (393.9 ± 18.46 mg/g), followed by F2 (382.4 ± 18.46 mg/g) and F1 (364.8 ± 18.46 mg/g), which were markedly higher than the iron concentration in *Moringa oleifera* (0.70 mg/g) (USDA). Given that iron deficiency is a leading cause of anemia in women, the high iron content in this formulation is particularly beneficial in preventing anemia and supporting maternal health (Abbaspour *et al.,* 2014). The significantly elevated iron levels in the formulations indicate their potential role in combating iron-deficiency anemia, which is a major public health concern, particularly among children and pregnant women (McLean *et al.,* 2009).

Potassium regulates fluid balance, nerve function, and muscle contractions. The RDA for potassium is 2600 mg/day for women. Adequate intake helps reduce the risk of hypertension and cardiovascular diseases, which are concerns for pregnant and lactating women (National Academies of Sciences, 2019). As shown in (Table 3), Potassium content remained relatively stable across the formulations, with F1 (574.5 ± 34.56 mg/g), F2 (575.0 ± 34.56 mg/g), and F3 (573.7 ± 34.56 mg/g), all of which were considerably higher than the *Moringa oleifera* reference value of 1.32 mg/g (USDA). Potassium plays a vital role in maintaining electrolyte balance, nerve function, and cardiovascular health, helping to regulate blood pressure and reduce the risk of hypertension (Beto *et al.,* 2016). The high potassium content in this formulation supports electrolyte balance and overall cardiovascular health (Palmer, 2015).

Magnesium plays a vital role in muscle and nerve function, blood glucose control, and bone health. The RDA for magnesium is 310-320 mg/day for women and increases to 350-400 mg/day during pregnancy (National Institutes of Health, 2021). As illustrated in (Table 3), Magnesium levels were relatively consistent across the formulations, with F1 (141.4 ± 7.08 mg/g), F2 (140.9 ± 7.08 mg/g), and F3 (140.1 ± 7.08 mg/g), all of which exceeded the reported *Moringa oleifera* magnesium content of 0.36 mg/g (FAO). Magnesium is essential for enzymatic reactions, muscle and nerve function, and bone health, and its presence in adequate amounts in the formulations suggests potential benefits in preventing magnesium deficiency-related conditions such as muscle cramps and cardiovascular disorders (Volpe, 2013). Magnesium deficiency has been linked to pre-eclampsia, muscle cramps, and fatigue, making this formulation a beneficial

Table 4: Proximate composition of the formulation.

Parameters	F1	F2	F3	*M. oleifera*
Ash content	15.17±0.15[a]	14.97±0.15[a]	14.73±0.21[a]	9.00
Moisture content	8.00±0.10[a]	7.87±0.15[a]	7.77±0.15[a]	7.50
Lipids	8.47±0.15[b]	8.27±0.15[ab]	8.07±0.15[a]	8.00
Fibre	5.07±0.15[a]	4.97±0.15[a]	4.87±0.15[a]	8.50
Crude protein	23.23±0.21[a]	22.97±0.25[a]	22.77±0.25[a]	27.10
Carbohydrates	40.30±0.26[a]	39.97±0.25[a]	39.77±0.25[a]	38.20

Data are presented as mean ± SD for three replicate experiments. Means with different superscript letters within the same row are significantly different (P< 0.05; One-Way ANOVA with Tukey's post hoc test). A significant difference between the two groups of sample and standard determined using an independent T-test at P < 0.05.

supplement for women's reproductive health (Rosanoff *et al.,* 2012).

Zinc is essential for immune function, wound healing, and reproductive health. The RDA for zinc is 8 mg/day for women and 11 mg/day during pregnancy (WHO, 2021). According to (Table 3), Zinc content showed minor variations, with F1 (20.2 ± 1.05 mg/g), F2 (20.0 ± 1.05 mg/g), and F3 (19.8 ± 1.05 mg/g), all significantly higher than the reported *Moringa oleifera* zinc content of 0.60 mg/g (USDA). Zinc is crucial for immune function, wound healing, and cellular metabolism, and the relatively high levels observed in the formulations suggest their potential role in enhancing immune response and preventing zinc deficiency-related disorders such as impaired growth and weakened immunity (Prasad, 2013). Zinc deficiency can impair immune response and fetal development, making this formulation a supportive dietary supplement for maternal and child health (King, 2018).

The formulated micronutrient powders exhibited higher concentrations of calcium, iron, potassium, magnesium, and zinc compared to *Moringa oleifera*, indicating their potential to serve as effective dietary supplements for addressing mineral deficiencies and improving overall health outcomes in women of reproductive age.

The proximate composition of the formulated micronutrient powders varied slightly across the three formulations (F1, F2, and F3). The ash content as shown in (Table 4) indicates that the total mineral composition, ranged from 14.7% (F3) to 15.2% (F1). These values were higher than the 9.0% reported for *Moringa oleifera* (FAO), suggesting an enhanced mineral profile due to the inclusion of additional natural ingredients.

As shown in (Table 4), Moisture content values were relatively low, with F1, F2, and F3 recording 8.0%, 7.9%, and 7.8%, respectively. These values were comparable to the 7.5% reported for *Moringa oleifera* (USDA), indicating good shelf stability, as lower moisture content reduces the risk of microbial growth and spoilage (FAO, 2021).

As shown in Table 4, the lipid content across the formulations was found to be 8.4% (F1), 8.3% (F2), and 8.1% (F3), aligning closely with the 8.0% recorded in *Moringa oleifera* (FAO). This suggests that the formulated powders retained a beneficial lipid composition, which is essential for energy provision and fat-soluble vitamin absorption (FAO, 2020).

Dietary fiber levels were observed to be within 4.87% to 5.05%, with the highest value recorded in F1 and the lowest in F3 as shown in (Table 4). Compared to *Moringa oleifera*, which contains 8.5% fiber (USDA), the formulations exhibited slightly lower fiber content. Despite this, the fiber levels remain within an acceptable range for promoting digestive health and enhancing satiety (USDA, 2022).

According to (Table 4), the crude protein content across the formulations was relatively high, with values of 23.2% (F1), 23.0% (F2), and 22.8% (F3). These values, although lower than the 27.1% found in *Moringa oleifera* (USDA), indicate that the formulated powders provide a substantial protein source, which is essential for growth, muscle maintenance, and enzymatic functions (FAO, 2021).

Carbohydrate content was observed to be 40.2% in F1, 40.0% in F2, and 39.8% in F3 as shown in (Table 4). These values were slightly higher than the 38.2% reported for *Moringa oleifera* (FAO), suggesting that the formulations could serve as an efficient energy source, which is vital for metabolic processes and daily bodily functions (FAO, 2020).

The proximate composition of the formulated micronutrient powders demonstrates their potential as a nutritionally balanced supplement. The variations among the formulations reflect differences in ingredient proportions, with all formulations maintaining a favorable nutrient profile that aligns well with standard dietary requirements.

The storage stability of the formulated micronutrient powder was evaluated based on peroxide value, free fatty acid content, and moisture content. These parameters are essential indicators of oxidative stability, hydrolytic rancidity, and product shelf life (Górnaś *et al.,* 2016). The storage stability of the formulated micronutrient powder is crucial in determining its shelf life, quality, and effectiveness in delivering essential nutrients. The peroxide value is an indicator of lipid oxidation, which affects the rancidity and overall quality of the formulation. According to Codex Alimentarius standards, peroxide values for stable food products should remain below 10

Table 5: Storage stability of the formulated powder.

Parameters	F1	F2	F3	*M. oleifera*
Peroxide value	8.73±0.21[a]	8.40±0.20[a]	8.30±0.20[a]	4.50
Free fatty acid	0.93±0.01[b]	0.91±0.02[ab]	0.89±0.02[a]	0.80
Moisture content	8.00±0.10[a]	7.87±0.15[a]	7.77±0.15[a]	8.00

Data are presented as mean ± SD for three replicate experiments. Means with different superscript letters within the same row are significantly different (P< 0.05; One-Way ANOVA with Tukey's post hoc test). A significant difference between the two groups of sample and standard determined using an independent T-test at P < 0.05.

Table 6: Antinutritional factor contents of the formulation.

Anti-Nutrients	F1	F2	F3	M. *oleifera*
Nitrate	82.07±0.49[a]	85.77±0.42[b]	86.33±0.31[b]	80.00[b]
Tanins	21.20±0.26[a]	20.83±0.31[a]	20.57±0.25[a]	20.00[b]
Cyanides	5.73±0.12[a]	5.57±0.15[a]	5.47±0.15[a]	5.00[b]
Phytates	1.91±0.02[b]	1.88±0.02[ab]	1.86±0.03[a]	1.90[a]
Oxalate	0.0028±0.00[a]	0.0027±0.00[a]	0.0026±0.00[a]	0.0030[b]

Data are presented as mean ± SD for three replicate experiments. Means with different superscript letters within the same row are significantly different (P< 0.05; One-Way ANOVA with Tukey's post hoc test). A significant difference between the two groups of sample and standard determined using an independent T-test at P < 0.05.

meq/kg to ensure freshness (FAO/WHO, 2019). As shown in (Table 4), the peroxide value, which measures the extent of lipid oxidation, was found to decrease slightly over time. The values recorded were 8.7 ± 0.44 meq/kg, 8.4 ± 0.44 meq/kg, and 8.3 ± 0.44 meq/kg for formulations F1, F2, and F3, respectively. The observed decrease could be attributed to the presence of antioxidants, such as vitamin E, which may have played a role in delaying lipid peroxidation (Shahidi and Zhong, 2015). However, the peroxide values remained within the acceptable limits for food products, indicating good oxidative stability.

Free fatty acid (FFA) content is an important parameter in assessing lipid degradation and hydrolysis. It is an indicator of lipid hydrolysis and potential rancidity. The values ranged from 0.93 ± 0.05% in F1 to 0.89 ± 0.05% in F3 according to (Table 5). This reduction suggests minimal enzymatic or microbial degradation during storage, which may be due to the low moisture content and proper packaging of the product (Rani *et al.,* 2018). The acceptable FFA level for food-grade powders is generally below 1% and the formulations is within the permissible range and is unlikely to exhibit off-flavors or undesirable rancidity, provided proper storage is maintained (Choe and Min, 2006). Moisture content plays a crucial role in microbial stability and powder flowability. For powdered food formulations, ideal moisture content should be below 10% to reduce microbial growth and clumping issues (Barbosa-Cánovas *et al.,* 2005). Table 5 shows that that moisture content was maintained within a stable range, with values of 8.0 ± 0.32%, 7.9 ± 0.32%, and 7.8 ± 0.32% for F1, F2, and F3, respectively. The slight reduction in moisture content may have contributed to the prevention of microbial growth and enzymatic activities that could lead to deterioration (Barbosa-Cánovas *et al.,* 2005). The moisture levels observed were within acceptable ranges for powdered food products, ensuring extended shelf life and stability.

The storage stability assessment suggests that the micronutrient powder formulations exhibited good resistance to oxidative deterioration, lipid hydrolysis, and moisture-related degradation. This stability is crucial for maintaining the nutritional and sensory properties of the product over time (Ahmed *et al.,* 2016).

Antinutritional factors are naturally occurring compounds in plant-based foods that can interfere with nutrient absorption and utilization. The presence of antinutritional factors such as nitrates, tannins, cyanide, phytate, and oxalate in food products can influence their bioavailability and potential health effects. In the formulated micronutrient powder, the levels of these compounds were analyzed to assess their potential impact on nutrient absorption and overall nutritional quality.

From (Table 6), Nitrate content in the samples ranged from 82.0 ± 4.60 to 86.3 ± 4.60 mg/kg. Although nitrates are naturally occurring in vegetables and other plant-based foods, their excessive intake has been associated with methemoglobinemia and potential carcinogenic effects due to the formation of N-nitroso compounds (Santamaria, 2006). However, the detected nitrate levels remain within acceptable limits and do not pose a significant health risk (EFSA, 2008).

As shown in (Table 6), Tannins were found in concentrations between 20.6 ± 2.56 and 21.2 ± 2.56 mg/kg. Tannins are known to interfere with the absorption of proteins and minerals such as iron and zinc by forming insoluble complexes (Khokhar and Apenten, 2003). Despite this, the moderate levels observed in the present study suggest that their impact on micronutrient

Table 7: Sensory evaluation of the formulated powder.

Parameters	F1	F2	F3
Color	8.33±0.58^{b}	7.67±0.58^{ab}	6.67±0.58^{a}
Appearance	8.67±0.58^{b}	7.67±0.58^{ab}	6.67±0.58^{a}
Texture	7.67±0.58^{b}	6.67±0.58^{ab}	5.67±0.58^{a}
Taste	8.33±0.58^{b}	7.33±0.58^{ab}	6.33±0.58^{a}
Consistency	8.67±0.58^{b}	7.67±0.58^{ab}	6.67±0.58^{a}
Flavor	7.33±0.58^{b}	6.33±0.58^{ab}	5.33±0.58^{a}

Data are presented as mean ± SD for three replicate experiments. Means with different superscript letters within the same row are significantly different (P< 0.05; One-Way ANOVA with Tukey's post hoc test).

bioavailability may be minimal when consumed as part of a balanced diet (Shahidi and Naczk, 2004).

Cyanide levels ranged from 5.47 ± 0.39 to 5.73 ± 0.39 mg/kg as shown in (Table 6). Cyanogenic glycosides, which release hydrogen cyanide upon metabolism, can be toxic in high amounts (FAO/WHO, 1995). However, the levels detected in the micronutrient powder are significantly lower than the established toxic threshold, indicating minimal risk of cyanide poisoning from consumption.

From (Table 6), Phytate content was recorded between 1.86 ± 0.10 and 1.91 ± 0.10 mg/kg. Phytic acid is an antinutrient that binds essential minerals such as calcium, iron, and zinc, reducing their bioavailability (Hurrell, 2003). The relatively low levels in the formulation suggest that phytate-induced mineral deficiency is unlikely, particularly when paired with dietary strategies such as fermentation or soaking to reduce phytate content (Lopez *et al.,* 2002). As further shown in (Table 6), Oxalate concentrations were found between 0.00263 ± 0.00014 and 0.00285 ± 0.00014 mg/kg. Oxalates can chelate calcium and other minerals, leading to reduced absorption and potential kidney stone formation in susceptible individuals (Noonan and Savage, 1999). The values reported in this study are low and comparable to levels found in commonly consumed leafy vegetables, indicating a minimal risk of adverse effects.

Overall, the levels of antinutritional factors in the micronutrient powder remain within safe limits and are unlikely to pose significant health concerns. The formulation appears to provide an adequate balance between essential micronutrients and naturally occurring inhibitors, supporting its potential as a nutritionally beneficial supplement.

Sensory evaluation is a critical aspect of food formulation as it assesses consumer acceptance of food products based on attributes such as color, appearance, texture, taste, consistency, and flavor (Meilgaard *et al.,* 2016). The formulated powder exhibited an army green color, which is likely attributed to the presence of moringa and spinach. Green-colored foods are generally associated with health benefits (Pathare *et al.,* 2013).

Color influences consumer perception and acceptability. From (Table 7), the powder had a high color rating (8.33 to 6.67), similar to values reported in similar formulations (Ibrahim *et al.,* 2018). The slight decline in color scores may be attributed to the natural pigmentation of ingredients like moringa and carrot.

Appearance is a crucial determinant of acceptability. Scores ranged from 8.67 to 6.67 according to (Table 6), suggesting good visual appeal. A study by Ogunsina *et al.* (2019) found comparable results in micronutrient-enhanced food products.

From (Table 7), Texture ratings (7.67 to 5.67) indicate good mouthfeel but slight variations due to formulation differences. This aligns with previous studies on plant-based powders (Adebayo and Oke, 2020).

Taste plays a major role in consumer acceptance. The agreeable taste of the formulation indicates that the combination of ingredients resulted in a palatable product. From (Table 7), Taste ranges from 8.33 to 6.33 which confirm the product's palatability, with higher scores observed in formulations with balanced ingredient ratios. This is consistent with studies on fortified foods (Oladiran and Emmanuel, 2021). A uniform consistency ensures a homogeneous distribution of nutrients and enhances reconstitution properties when mixed with liquids. This is an important quality parameter for ensuring product integrity and stability during storage (Szczesniak, 2002). Consistency ratings 8.67 to 6.67 as shown in (Table 7) suggest good reconstitution properties. Similar results were observed in previous sensory studies on food powders (Olorode *et al.,* 2014).

The predominant leaf flavor is attributed to the natural components of moringa and spinach. While some consumers associate leafy flavors with nutritional benefits, others may find them overpowering. According to (Table 7), Flavor scores (7.33 to 5.33) indicate acceptable but variable flavor perception. Studies have shown that ingredient interaction may influence flavor retention (Blok, Bolhuis and Stieger, 2020). Future research can explore flavor-masking techniques or complementary ingredient pairings to enhance overall sensory appeal (Tuorila and Monteleone, 2009).

The formulation was rated as consumable, indicating that the sensory attributes were within an acceptable range for most consumers. This suggests that while there is room for improvement, the product has the potential to be well received in its target demographic, particularly among health-conscious individuals (Lawless and Heymann, 2010).

Table 8: Functional properties of the formulated powder.

Parameters	F1	F2	F3	M. *oleifera*
Bulk density	0.50±0.02[a]	0.49±0.02[a]	0.48±0.02[a]	0.45
Water adsorption capacity	1.30±0.05[a]	1.28±0.06[a]	1.26±0.06[a]	1.25
Swelling capacity	1.35±0.05[a]	1.35±0.06[a]	1.32±0.07[a]	1.20
Viscosity	79.33±0.85[a]	79.13±1.19[a]	78.40±1.35[a]	85.00
PH	6.17±0.15[a]	6.13±0.21[a]	6.03±0.21[a]	5.80

Data are presented as mean ± SD for three replicate experiments. Means with different superscript letters within the same row are significantly different (P< 0.05; One-Way ANOVA with Tukey's post hoc test). A significant difference between the two groups of sample and standard determined using an independent T-test at P < 0.05.

The sensory evaluation results suggest that the formulated micronutrient powder has favorable sensory attributes, particularly in terms of texture, taste, and consistency. However, certain attributes such as color intensity and leaf flavor may require further refinement to enhance consumer acceptability. Future formulation improvements could explore natural flavor enhancers, color-balancing techniques, and blending strategies to optimize overall sensory appeal.

Functional properties play a crucial role in determining the quality, usability, and acceptability of food formulations. These properties influence the behavior of food ingredients during processing, storage, and consumption (Olawoye *et al.,* 2020).

Bulk density affects packaging, transportation, and storage of food powders. From (Table 8), the bulk density values of the micronutrient powder ranged from 0.48 to 0.50 g/cm³. These values are comparable to those reported for similar plant-based powders (Olorode *et al.,* 2014). A higher bulk density suggests better compactness, which is advantageous for storage efficiency.

Water Absorption Capacity (WAC) determines the ability of food powders to retain moisture, which influences texture and rehydration properties. The WAC values ranges between 1.26 to 1.30 g/g from (Table 8) and this align with findings from studies on plant-based powders (Oladiran and Emmanuel, 2021). This property is particularly important for reconstituted products.

Swelling capacity determines how well the powder can increase in volume upon hydration, influencing texture and mouthfeel. The results in (Table 8) ranging from 1.32 to 1.35 g/g indicate good swelling potential, which is beneficial for instant food applications. Similar swelling capacities were reported by Nwosu *et al.* (2022) in moringa-based food formulations.

Viscosity is an important factor in determining the textural and flow characteristics of food products. The measured viscosity values as shown in (Table 8) is 79.33, 79.13 and 78.40 for F1, F2 and F3 respectively and this suggest moderate thickness, suitable for nutrient-dense formulations (Blok, Bolhuis and Stieger, 2020). The values indicate moderate viscosity, meaning the formulation provides a desirable consistency without excessive thickness, which is important for consumer preference (Barbosa-Cánovas, *et al.,* 2005). The pH of a food product influences its stability, taste, and microbial susceptibility. The recorded pH values (6.0 to 6.2) in (Table 8) indicate a near-neutral pH, suggesting mild acidity. This is desirable for stability and microbial safety (Adebayo and Oke, 2020). The pH values fall within the neutral range, indicating that the formulation is neither highly acidic nor basic, making it versatile for incorporation into various food systems (García *et al.,* 2019).

The functional properties of the formulated micronutrient powder suggest its suitability for diverse food applications. Its moderate bulk density, water absorption, and swelling capacity make it adaptable for different formulations, while its viscosity and pH support stability and consumer acceptability. Further optimization may involve modifications to enhance specific functional characteristics for targeted applications.

Conclusion

This study successfully developed and optimized a micronutrient powder blend using *Moringa oleifera*, spinach, carrot, and eggshell, demonstrating its potential as a nutrient-dense dietary supplement. The comprehensive micronutrient analysis confirmed that the formulations provided significant levels of essential vitamins and minerals, surpassing the values found in *Moringa oleifera* alone. Notably, the formulations exhibited enhanced concentrations of vitamin A, vitamin E, folic acid, calcium, and iron - nutrients critical for women of reproductive age. These findings highlight the formulation's potential in addressing micronutrient deficiencies, particularly in populations vulnerable to anemia, osteoporosis, and reproductive health complications.

The proximate composition and functional properties of the powder further underscore its nutritional and technological viability. The formulations maintained an optimal balance of macronutrients, with adequate protein, fiber, and lipid content to support overall dietary needs. Low moisture levels contributed to extended shelf stability, while

favorable bulk density, water absorption capacity, and swelling index confirmed its suitability for various food applications. The powder also demonstrated good oxidative and hydrolytic stability, ensuring minimal nutrient degradation over time. Sensory evaluation results revealed high acceptability in terms of texture, consistency, and overall palatability, though minor adjustments in color intensity and flavor balance could enhance consumer preference. Additionally, the antinutritional factor analysis indicated that compounds such as phytates, tannins, and oxalates were present at safe levels, ensuring that mineral bioavailability was not significantly compromised. These findings position the formulated micronutrient powder as a promising intervention for combating micronutrient deficiencies, particularly in resource-limited settings where access to diverse diets is constrained. Future research should explore bioavailability studies and clinical trials to further validate its efficacy. Additionally, large-scale production and commercialization efforts could focus on optimizing formulation strategies to enhance both nutritional impact and consumer appeal. Overall, this research contributes valuable insights into sustainable, food-based strategies for improving micronutrient intake, aligning with global nutrition and public health objectives.

Recommendations

Given the findings of this study, several key recommendations are proposed to enhance the potential impact of the developed micronutrient powder. These recommendations are made to ensure effective utilization, policy support, and further research that will facilitate the widespread adoption and long-term sustainability of the product. Addressing micronutrient deficiencies requires a multi-faceted approach that integrates production scalability, consumer education, and regulatory support. Thus, it is therefore recommended that:

1. Efforts should be made to scale up production and explore commercial viability, ensuring that the powder blend can reach wider populations, particularly in rural and underserved communities.
2. Additional studies should be conducted to assess the bioavailability and long-term health impacts of the micronutrient powder when incorporated into regular diets.
3. Policymakers should consider supporting initiatives that promote the local production of nutrient-dense food supplements. Subsidies and incentives could encourage widespread adoption.
4. Public health campaigns should be organized to educate communities about the benefits of consuming fortified food products, emphasizing the role of micronutrient powders in preventing deficiencies.
5. Future research should explore variations of the formulation with additional ingredients that may further enhance the nutritional profile and cater to specific dietary needs.
6. The micronutrient powder should be considered for inclusion in school feeding programs, maternal health initiatives, and emergency nutrition interventions.

Conflict of Interest

The authors declared no conflict of interest.

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