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Analysis of Micronutrients and Organoleptic Acceptability of a Functional Biscuit Formulation Made with Moringa Flour and Mackerel

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ABSTRACT

Micronutrient deficiencies, particularly of iron, zinc, calcium, and vitamin A, remain a persistent public health problem in Indonesia and contribute to stunting and anemia in children. Biscuits are a widely consumed snack and offer a practical vehicle for food-based fortification using local ingredients. This study aimed to analyze the micronutrient content and organoleptic acceptability of a functional biscuit formulated with moringa leaf flour (Moringa oleifera) and mackerel flour (Rastrelliger kanagurta). A completely randomized experimental design was applied with four formulations: F0 (control, 100% wheat flour), F1 (5% moringa flour + 10% mackerel flour), F2 (7.5% + 15%), and F3 (10% + 20%), each in three replicates. Iron, zinc, and calcium were determined by atomic absorption spectrophotometry, and β-carotene by high-performance liquid chromatography. A hedonic test involving 30 semi-trained panelists assessed color, aroma, taste, texture, and overall acceptability. Data were analyzed using one-way ANOVA with Duncan's test and the Kruskal–Wallis test. Increasing substitution levels significantly raised all four micronutrients ($p < 0.05$), with F3 showing the highest values. In contrast, hedonic scores decreased as substitution increased, mainly due to the green color and fishy aroma. Based on the effectiveness index, F2 was the best formulation, balancing micronutrient enrichment and consumer acceptance.

INTRODUCTION

Nutritional problems in Indonesia are not only energy and protein deficiencies, but also micronutrient deficiencies, often referred to as hidden hunger. Iron, zinc, calcium, and vitamin A deficiencies are not always clinically apparent, but their impact on children's linear growth, cognitive function, and immune system is long-term. National serum biomarker-based data shows that school-age children in Indonesia still experience deficiencies of ferritin (13.4%), zinc (19.7%), calcium (4.2%), vitamin A (3%), and vitamin D (12.7%) (Ernawati et al., 2023). The same study found that serum zinc and vitamin A levels were significantly lower in stunted children compared to children with

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normal nutritional status, suggesting that micronutrient deficiencies and impaired linear growth are closely linked (Ernawati et al., 2023).

This relationship explains why stunting reduction is not simply about increasing calorie intake. Indonesian children's dietary intake is still dominated by cereals, while consumption of animal-based foods such as fish, meat, and eggs is relatively low, even though animal-based foods are sources of iron and zinc with high bioavailability (Ernawati et al., 2023). Therefore, a food-based approach that combines local plant and animal sources is considered more sustainable than single supplementation because it is cheaper, culturally acceptable, and can be produced locally.

One of the most widely studied local plant ingredients is the Moringa oleifera leaf. Moringa leaves are known to be rich in protein, iron, calcium, carotenoids as precursors of vitamin A, and vitamin C, which aids the absorption of non-heme iron (Rotella et al., 2023). A study in Indonesia concluded that the addition of Moringa leaf powder significantly increased the protein, fiber, and mineral content of food products, but the acceptable concentration for consumers was generally no more than 5% (Angelina et al., 2021). Intervention evidence also demonstrates the clinical benefits of Moringa. Moringa supplementation in pregnant women is associated with a reduced risk of stunting in children aged 36–42 months (Basri et al., 2021), and biscuits made from Moringa leaf flour have been reported to improve anemia in pregnant women (Mangul et al., 2021).

On the other hand, mackerel (Rastrelliger kanagurta) is a small, inexpensive pelagic fish, readily available in almost all Indonesian waters, and rich in high-quality protein, bone calcium, and omega-3 fatty acids. Processing mackerel into flour allows this ingredient to be substituted in cereal products without drastically changing the product's form. Nadimin et al. (2021) showed that substituting mackerel flour in biscuits increased nutritional content and remained acceptable to panelists. Recent evidence even shows that mackerel nuggets fortified with 7% moringa leaf flour improved height-for-age (H/A) and weight-for-age (W/A) z-scores in stunted toddlers in West Nias (Haruni et al., 2025). These findings indicate that the combination of moringa and fish is complementary: moringa contributes nonheme iron, calcium, and β-carotene, while fish contributes protein, zinc, and calcium with better bioavailability.

Biscuits were chosen as a fortification vehicle because they have a low water content, long shelf life, easy distribution, and are preferred by all ages. Biscuits have also been widely used in Supplementary Feeding (PMT) programs in Indonesia. Several international studies have formulated biscuits or cookies enriched with moringa. Hedhili et al. (2021) reported that moringa supplemented biscuits increased nutritional value, but acceptance varied across consumer segments. Riaz and Wahab (2021) and Salama et al. (2022) found increased mineral and protein content in wheat biscuits enriched with moringa leaf powder, accompanied by decreased sensory scores at high substitution levels. Kc et al. (2022) compared moringa flower and leaf powder, while Agba et al. (2024) tested decolorized moringa leaf powder to address the green color and bitter taste issues.

Other studies have combined moringa with local sources of carbohydrates or plant proteins. Moringa has been combined with sorghum and sesame flour (Oboulbiga et al., 2026), rice bran (Lisnawati et al., 2026), multigrain (Anjum et al., 2025), millet (Bhuyan, 2025), buckwheat (Kaushik et al., 2023), pigeon peas (Nnadi et al., 2025), and soy flour and tempeh (Lestari et al., 2024). In Indonesia, purple sweet potato and moringa biscuits were developed as snacks for toddlers at risk of stunting (Rahman et al., 2025), and moringa flour biscuits were also proposed as a child supplement (Coelho et al., 2022). A

national literature review confirmed that the acceptability of moringa products is largely determined by the level of addition and processing techniques (Rohman & Rahmawaty, 2024).

The combination of moringa and fish has also begun to be studied. Sopandi and Hakiki (2024) formulated cookies with snakehead fish flour and moringa as a high-protein functional food. Hartati et al. (2024) developed biscuits made from catfish, purple sweet potato, and moringa flour for supplementary feeding to toddlers, while Rumapea et al. (2025) created snack bars from moringa and lumi-lumi fish. However, these studies generally focused on protein, macronutrients, or proximate nutrients, and used freshwater fish or fish with limited distribution.

Based on this research, three research gaps emerged. First, there is no biscuit formulation that specifically combines moringa leaf flour and mackerel flour, even though mackerel is one of the most affordable and widely available marine fish in coastal areas of Indonesia. Second, most moringa biscuit studies report protein and proximate levels, while simultaneous analysis of micronutrients relevant to stunting—iron, zinc, calcium, and beta-carotene—is still rare. Third, previous studies generally assessed nutritional content and acceptability separately, without an objective method to determine the formulation that best balances nutritional value and consumer acceptability.

The novelty of this research lies in three things. (1) This research is the first functional biscuit formulation that combines moringa leaf flour and mackerel fish flour with a tiered substitution ratio of 1:2, so that plant and animal micro sources work complementary. (2) The analysis focuses on the profile of four key micronutrients for preventing stunting and anemia, accompanied by calculations of their contribution to the child's Nutritional Adequacy Intake (AKG) per serving. (3) Determination of the best formulation is carried out using the De Garmo effectiveness index which integrates chemical and sensory parameters simultaneously, so that the results can be directly used as a basis for developing local food-based PMT.

Based on the description, this study aims to: (1) analyze the levels of iron, zinc, calcium, and β-carotene in biscuits with various levels of substitution of moringa leaf flour and mackerel fish flour; (2) evaluate the organoleptic acceptability of biscuits based on the attributes of color, aroma, taste, texture, and overall acceptability; and (3) determine the best formulation that balances micronutrient enrichment and consumer acceptability.

RESEARCH METHODS

A. Research Design

This study was a laboratory experiment with a completely randomized design (CRD) with one factor, namely the substitution level of wheat flour with a mixture of moringa leaf flour and mackerel fish flour. There were four treatments with three replications, resulting in 12 experimental units. The overall research flow is presented in Figure 1.

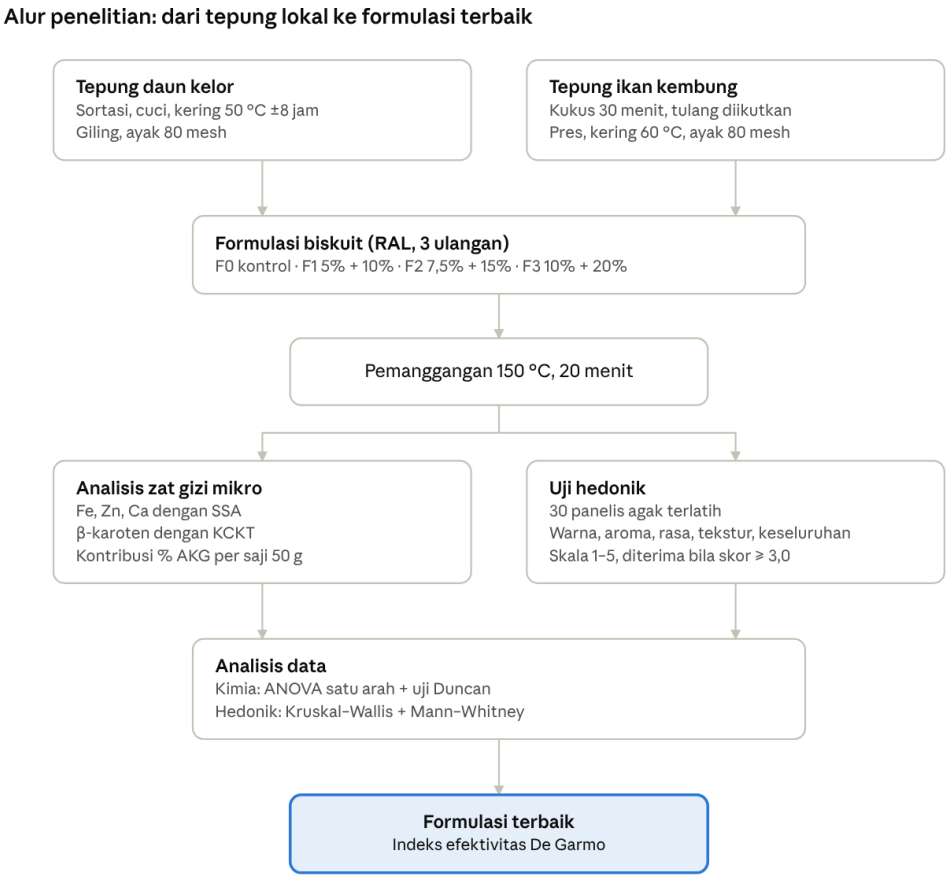


Figure 1: Research flow for the formulation of moringa–mackerel biscuits

The research flow, starting from the manufacture of two local flours, formulation and baking, chemical and sensory tests in parallel, to determining the best formulation.

B. Materials and Tools

The main ingredients consist of fresh Moringa leaves (3rd to 7th leaves from the top), fresh mackerel (Rastrelliger kanagurta), low protein wheat flour, margarine, refined sugar, egg yolk, powdered milk, and baking powder. Analytical chemicals include concentrated HNO_3, $HClO_4$, standard solutions of Fe, Zn, and Ca, standard β-carotene, and analytical grade hexane and acetone solvents. The tools used include a cabinet dryer, disc mill, 80 mesh sieve, baking oven, atomic absorption spectrophotometer (AAS), and high performance liquid chromatography (HPLC) with a UV-Vis detector.

C. Making Moringa Leaf Flour and Mackerel Flour

Moringa leaves were sorted, washed, drained, and then dried in a cabinet dryer at 50°C for approximately 8 hours until the moisture content was below 10%. Low-temperature drying was chosen to minimize the destruction of β-carotene. The dried leaves were ground and sieved through an 80-mesh sieve.

The mackerel was gutted, washed, and steamed at approximately 100°C for 30 minutes to reduce the fishy odor and facilitate meat separation. The bones were left in place to maintain the calcium content. The meat and bones were then pressed to reduce the fat and water content, dried at 60°C for approximately 10 hours, ground, and sieved through an 80-mesh sieve.

D. Biscuit Formulation

The formulation was prepared with a 1:2 ratio of moringa flour to mackerel flour, based on preliminary testing and the reported acceptance limit of moringa flour of approximately 5–10% in the literature (Angelina et al., 2021; Rohman & Rahmawaty, 2024). The substitution percentage was calculated against the total flour (Table 1).

Table 1
Biscuit formulation per 100 g total flour

Material	F0	F1	F2	F3
Wheat flour (g)	100	85	77.5	70
Moringa leaf flour (g)	0	5	7.5	10
Mackerel flour (g)	0	10	15	20
Margarine (g)	50	50	50	50
Powdered sugar (g)	40	40	40	40
Egg yolk (g)	20	20	20	20
Milk powder (g)	10	10	10	10
Baking powder(g)	1	1	1	1

Source: Research data (processed)

Margarine and sugar were beaten until smooth, then egg yolks were added. A mixture of flour, powdered milk, and baking powder was gradually added until a smooth dough formed. The dough was flattened to a thickness of approximately 4 mm, shaped, and baked at 150°C for 20 minutes. The biscuits were cooled and packaged in airtight containers before analysis.

E. Micronutrient Analysis

The levels of iron (Fe), zinc (Zn), and calcium (Ca) were determined by the wet digestion method using HNO_3 and $HClO_4$, then measured by SSA at wavelengths of 248.3 nm (Fe), 213.9 nm (Zn), and 422.7 nm (Ca). β-carotene levels were extracted with a hexane–acetone mixture and analyzed by HPLC at a wavelength of 450 nm. Vitamin A values were expressed as Retinol Activity Equivalent (RAE) with a conversion factor of 12 µg β-carotene = 1 µg RAE. All analyses were performed in duplicate for each replication and expressed per 100 g of biscuits.

Contribution to the AKG is calculated for a serving size of 50 g biscuits with the reference AKG for children aged 4–6 years according to the Indonesian Minister of Health Regulation Number 28 of 2019, namely Fe 10 mg, Zn 5 mg, Ca 1,000 mg, and vitamin A 450 µg.

F. Organoleptic Test

The hedonic test was conducted by 30 semi-trained panelists, nutrition students aged 19–23 years who did not have fish allergies and were willing to sign an informed consent form. Panelists rated color, aroma, taste, texture, and overall acceptability using a five-point hedonic scale (1 = dislike very much; 5 = like very much). Samples were assigned random three-digit codes and served in random order. Panelists were asked to neutralize their taste buds with mineral water between samples. Products were deemed acceptable if the average score was ≥ 3.0 (neutral to like).

G. Data Analysis

Micronutrient data were analyzed using one-way ANOVA at a 5% significance level, followed by Duncan's Multiple Range Test if significant differences were found. Hedonic data were ordinal in nature, therefore analyzed using the Kruskal–Wallis test and

then the Mann–Whitney test. The best formulation was determined using the De Garmo effectiveness index, with parameter weights determined by the panelists. The analysis was performed using SPSS version 26 software.

RESULTS AND DISCUSSION

A. Micronutrient Content of Biscuits

Substituting moringa leaf flour and mackerel fish flour significantly increased the levels of all four micronutrients ($p < 0.05$). The largest increases occurred in calcium and β-carotene, while the increases in iron and zinc were more moderate. The results of the analysis are presented in Table 2.

Table 2
Micronutrient content of biscuits per 100 g (mean ± SD, n = 3)

Formulation	Fe (mg)	Zn (mg)	Ca (mg)	β-carotene (µg)	Vitamin A (µg RAE)
F0 (control)	1.62 ± 0.08^{a}	0.68 ± 0.04^{a}	32.5 ± 1.8^{a}	18 ± 2^{a}	1.5
F1 (5% moringa + 10% fish)	2.21 ± 0.10^{b}	0.84 ± 0.05^{b}	104.6 ± 4.2^{b}	212 ± 11^{b}	17.7
F2 (7.5% moringa + 15% fish)	2.58 ± 0.11^{c}	0.93 ± 0.04^{c}	141.3 ± 5.1^{c}	318 ± 14^{c}	26.5
F3 (10% moringa + 20% fish)	3.07 ± 0.13^{d}	1.04 ± 0.06^{d}	179.8 ± 6.4^{d}	421 ± 17^{d}	35.1

Source: Research data (processed)

Iron levels increased from 1.62 mg in F0 to 3.07 mg in F3, nearly doubling. The largest contribution came from moringa leaf flour, known as one of the richest plant-based sources of iron (Rotella et al., 2023). A similar pattern was reported in whole wheat biscuits enriched with moringa leaf powder, where mineral levels increased with the addition level (Riaz & Wahab, 2021; Salama et al., 2022). The iron in moringa is non-heme iron, whose absorption can be inhibited by phytate and polyphenols. However, the presence of fish protein in this formulation has the potential to enhance non-heme iron absorption through the meat-fish-poultry factor effect. This is an advantage of the combination of moringa and fish compared to biscuits enriched only with moringa.

Zinc levels increased from 0.68 mg to 1.04 mg per 100 g. This increase in zinc content was relatively small because the zinc content in both ingredients is not as high as iron or calcium. Nevertheless, this increase is significant because zinc deficiency is the microdeficiency with the highest prevalence in school-age children in Indonesia, at 19.7%, and serum zinc levels are positively correlated with height/age z-scores (Ernawati et al., 2023). Zinc from fish has higher bioavailability than zinc from cereals, so its functional contribution is likely greater than indicated by absolute values.

Calcium showed the sharpest increase, from 32.5 mg to 179.8 mg per 100 g, or more than fivefold. This increase came from two sources: calcium-rich moringa leaves and mackerel flour made with bones. The decision to include fish bones in the flour production proved effective in increasing calcium without adding other ingredients. This finding aligns with Nadimin et al. (2021), who reported an increase in the nutritional content of biscuits by substituting mackerel flour, and Sopandi and Hakiki (2024), who found that the combination of fish flour and moringa produced cookies with higher nutritional value.

β-carotene levels increased from 18 µg in the control to 421 µg in F3. This increase was almost entirely due to moringa leaf flour, as mackerel is not a carotenoid source. The β-carotene content in biscuits was lower than theoretical estimates based on raw material content, as β-carotene is readily oxidized during drying and baking. Kc et al. (2022) and Agba et al. (2024) also noted that thermal processing alters the bioactive compounds profile of moringa in baked products. Therefore, the low baking temperature (150 °C) and leaf drying at 50 °C used in this study are appropriate for preserving provitamin A.

B. Contribution to Nutritional Adequacy Rates

To assess practical relevance, micronutrient content was converted to a percentage of the RDA for children aged 4–6 years per 50 g serving size (Table 3).

Table 3
Contribution of micronutrients per 50g serving to the RDA for children aged 4–6 years

Formulation	Fe (% RDA)	Zn (% RDA)	Ca (% RDA)	Vitamin A (% RDA)
F0	8.1	6.8	1.6	0.2
F1	11.1	8.4	5.2	2.0
F2	12.9	9.3	7.1	2.9
F3	15.4	10.4	9.0	3.9

Source: Research data (processed)

One serving of F2 provides 12.9% of the RDA for iron and 9.3% of the RDA for zinc, while F3 provides 15.4% and 10.4%, respectively. The iron contribution in F3 exceeds 15% of the RDA, a figure generally used as the threshold for nutrient source claims on food labels. Conversely, the vitamin A contribution remains low (below 4%) because some β-carotene is degraded and the conversion factor for β-carotene to retinol is relatively large. These findings suggest that moringa-mackerel biscuits are best positioned as a complementary source of iron, zinc, and calcium, rather than as a primary source of vitamin A. These results are consistent with the studies of Rahman et al. (2025) and Hartati et al. (2024) who developed moringa-based biscuits as a supplementary food for toddlers; both studies emphasize that the biscuits complement, rather than replace, a diverse diet.

C. Organoleptic Acceptability

In contrast to micronutrients, hedonic scores decreased with increasing substitution levels (Table 4). The Kruskal–Wallis test showed significant differences across all attributes ($p < 0.05$).

Table 4
Biscuit hedonic score (mean ± SD, n = 30, scale 1–5)

Attribute	F0	F1	F2	F3
Color	4.23 ± 0.63^{c}	3.87 ± 0.68^{bc}	3.53 ± 0.73^{b}	2.90 ± 0.80^{a}
Aroma	4.10 ± 0.66^{c}	3.83 ± 0.70^{bc}	3.47 ± 0.78^{b}	2.77 ± 0.82^{a}
Flavor	4.17 ± 0.59^{c}	3.97 ± 0.61^{bc}	3.63 ± 0.72^{b}	2.83 ± 0.87^{a}
Texture	4.03 ± 0.67^{b}	3.90 ± 0.66^{b}	3.77 ± 0.68^{b}	3.20 ± 0.76^{a}
Overall acceptance	4.20 ± 0.61^{c}	3.93 ± 0.64^{bc}	3.67 ± 0.71^{b}	2.87 ± 0.82^{a}
Panelists who accepted (score ≥ 3)	100%	96.7%	90.0%	63.3%

Source: Research data (processed)

Color was the attribute that declined most rapidly. Chlorophyll from moringa leaves imparted a brownish-green color after baking, which differed from panelists' expectations of golden-yellow biscuits. In F1, the color difference compared to the control was not significant, but in F3, the color score dropped below the acceptance threshold. This pattern aligns with the findings of Lestari et al. (2024) and Kaushik et al. (2023) that dark color is a major barrier to acceptance of moringa-enriched products. Agba et al. (2024) even used color-depleted moringa powder to address this issue, an approach that could be considered for further development.

Aroma and flavor are influenced by two sources simultaneously: the characteristic unpleasant aroma of moringa leaves and the fishy aroma of fishmeal. The combination of the two resulted in the F3 aroma score being the lowest among all attributes (2.77). Steaming the fish before drying helped reduce the fishy compounds, but not enough to achieve a 20% substitution level. Nadimin et al. (2021) and Rumapea et al. (2025) also reported that fish aroma is a determining factor in the acceptance of fishmeal-based products. The use of natural flavors such as vanilla, cinnamon, or pandan leaves can be a strategy to mask this aroma without reducing nutritional content.

Texture was the most stable attribute. Texture scores in F1 and F2 were not significantly different from the control, and only decreased in F3. This decrease was caused by the reduced proportion of gluten due to wheat substitution and the high protein and mineral content of fish meal, which made the biscuits harder and less crispy. Oboulbiga et al. (2026) showed that gluten-free biscuits enriched with moringa can still have good technological characteristics when the fat and water formulations are adjusted, so similar adjustments can be applied to this formulation.

Overall, F1 and F2 were still accepted by more than 90% of panelists, while F3 was only accepted by 63.3% of panelists, with an overall acceptance score below 3.0. This finding aligns with Angelina et al. (2021) and Rohman and Rahmawaty (2024) who stated that acceptance of moringa products declines sharply above a certain level. Hedhili et al. (2021) added that acceptance of moringa biscuits varies across consumer segments, so further testing on the target audience, namely children, is still needed.

D. Determining the Best Formulation

Previous analysis showed a trade-off: higher substitution rates result in higher micronutrient values but lower acceptability. The De Garmo effectiveness index was used to weigh both aspects simultaneously (Table 5).

Table 5
Effectiveness index value of biscuit formulation

Formulation	Micronutrient values (0.5g weight)	Organoleptic value (weight 0.5)	Effectiveness index	Ranking
F0	0.00	0.50	0.36	4
F1	0.19	0.43	0.62	2
F2	0.32	0.39	0.71	1
F3	0.50	0.08	0.58	3

Source: Research data (processed)

F2 achieved the highest effectiveness index (0.71), thus being designated the best formulation. Compared to the control, F2 increased iron by 59%, zinc by 37%, calcium by more than fourfold, and β-carotene by more than 17-fold, while remaining acceptable to 90% of panelists. F3 was nutritionally superior, but its low acceptability risked

reducing compliance when used in a PMT program. In the context of nutritional interventions, products with very high nutritional value will not be effective if not consumed regularly.

E. Implications and Limitations

The combination of moringa and mackerel has a strong scientific basis as a local food-based stunting prevention strategy. Interventions involving fish nuggets fortified with moringa flour have been shown to improve the anthropometric nutritional status of stunted toddlers (Haruni et al., 2025), and moringa supplementation during pregnancy has been associated with a reduction in stunting incidence (Basri et al., 2021). Moringa biscuits have also been reported to improve anemia in pregnant women (Manggul et al., 2021). With its affordable and widely available raw materials, the F2 formulation has the potential to be developed by food MSMEs or coastal community groups as a local PMT.

This study has several limitations. First, the hedonic test was conducted on adult panelists, so acceptability in children, the primary target group, requires separate testing. Second, the analysis did not include the in vitro bioavailability of iron and zinc, even though the phytate and polyphenol content of moringa can affect absorption. Third, the stability of β-carotene during storage has not been evaluated. Further research is recommended, including in vitro digestibility testing, shelf-life testing, and efficacy testing in children at risk of stunting.

CONCLUSION

Substituting wheat flour with a mixture of moringa leaf flour and mackerel fish meal significantly increased the iron, zinc, calcium, and β-carotene content of biscuits, and this increase was proportional to the level of substitution. Moringa was the primary contributor of iron and β-carotene, while mackerel meal and its bones were the primary contributors of calcium and zinc, thus complementing the two ingredients.

Organoleptic acceptability decreased with increasing substitution, particularly in color and aroma attributes, due to the green color from moringa chlorophyll and the fishy aroma from fish meal. Formulations F1 and F2 were still accepted by more than 90% of panelists, while F3 fell below the acceptance threshold.

Formulation F2 (7.5% moringa leaf flour + 15% mackerel fish flour) was the best formulation based on the effectiveness index. A 50 g serving of F2 provides approximately 13% of the RDA for iron and 9% of the RDA for zinc in children aged 4–6 years, making it suitable for development as a locally sourced food supplement to support the prevention of stunting and anemia. Further research is needed to test child acceptability, in vitro mineral bioavailability, shelf life, and the effectiveness of the biscuits through intervention trials.

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