International Journal of Public Health Science (IJPHS)
Vol. 15, No. 3, September 2026, pp. 839~847
ISSN: 2252-8806, DOI: 10.11591/ijphs.v15i3.27105

Red spinach flour substitution in cookies: effects on iron content, antioxidant activity, and sensory acceptability for anemia prevention

Yeni Tutu Rohimah[1], Dwi Retna Prihati[2], Titik Lestari[1]
[1]Department of Nursing, Poltekkes Kemenkes Surakarta, Surakarta, Indonesia
[2]Department of Midwifery, Poltekkes Kemenkes Surakarta, Surakarta, Indonesia

Article Info

Article history:

Received Apr 8, 2026
Revised Jul 8, 2026
Accepted Jul 12, 2026

Keywords:

Amaranthus tricolor L.
Antioxidants
Cookies
Iron
Sensory acceptability

ABSTRACT

Iron deficiency anemia affects approximately 30% of Indonesian women of reproductive age, underscoring the urgent need for affordable, iron-rich food interventions. This study investigated the effect of red *Amaranth* (*Amaranthus tricolor* L.) flour substitution on iron content, antioxidant activity, and sensory acceptability of composite flour cookies. A completely randomized design was employed with five substitution levels (0%, 10%, 20%, 30%, and 40%), each with three replications, yielding 15 experimental units of cookie batches. Iron content was determined using atomic absorption spectrophotometry, antioxidant activity via the DPPH method, and sensory acceptability through hedonic testing by 30 semi-trained panelists. Results demonstrated that iron content increased significantly ($p < 0.05$) with higher substitution levels, while antioxidant activity improved from very weak to moderate categories. However, sensory acceptability declined significantly at substitution levels of 30% and above, particularly in color and taste attributes. The 20% substitution level emerged as the optimal formulation, achieving substantially elevated iron content contributing meaningfully toward the recommended dietary allowance per serving, enhanced antioxidant capacity, and maintained good sensory acceptability across all attributes. These findings suggest that red *Amaranth*-substituted cookies represent a feasible, community-level dietary strategy for complementing existing iron deficiency anemia prevention programs in Indonesia.

Corresponding Author:

Yeni Tutu Rohimah
Department of Nursing, Poltekkes Kemenkes Surakarta
Surakarta, Indonesia
Email: yenitutur@gmail.com

1. INTRODUCTION

Indonesia faces a substantial burden of anemia, particularly among vulnerable populations. Data from the 2018 Indonesian Basic Health Research (*Riskesdas*) indicate that anemia prevalence among pregnant women reached 48.9%, while adolescent girls aged 15–24 years exhibited prevalence rates of approximately 32% [1]. These figures underscore the urgency of developing locally appropriate, food-based interventions. Indonesia's national strategy for anemia prevention has traditionally relied on iron supplementation programs; however, compliance remains low due to side effects and distribution challenges [1]. Consequently, food fortification and diversification using locally available ingredients have been advocated as complementary strategies [2].

Iron deficiency is one of the most common micronutrient problems worldwide, particularly in developing countries [3]. This condition can lead to iron deficiency anemia, which has serious impacts on the health, productivity, and quality of life of the community, particularly vulnerable groups such as children, adolescent girls, and pregnant women [4]. One strategy to address this problem is through food diversification by utilizing local foods rich in iron. Leafy green vegetables, including red amaranth (*Amaranthus tricolor* L.), are known to be excellent sources of minerals, including calcium, potassium, magnesium, and especially iron [5]. Red amaranth has the highest mineral index among various types of vegetables, surpassing other vegetables such as mustard greens, green spinach, and celery [6], [7].

Red spinach has a rich nutritional profile containing protein, carbohydrates, lipids (linoleic, oleic, and palmitic acids), and bioelements such as potassium, calcium, iron, and magnesium. Its iron content significantly exceeds that of wheat, green spinach, and meat, making it an excellent dietary supplement for anemia patients [8]. Furthermore, red spinach leaves are also an important source of antioxidants, including anthocyanins and carotenoids [9]. Research shows that red amaranth at the microgreen stage contains the highest total phenol content (84.74 mg/100 g) compared to other leafy vegetables such as green spinach (58.32 mg/100 g) [10]. Red spinach also contains vitamin A, vitamin C, and crude fiber which are beneficial for health [9].

Antioxidant activity is a critical consideration in functional food development. Green leafy vegetables, including amaranth, contain bioactive compounds with significant antioxidant capacity. Research on African green leafy vegetables demonstrates that antioxidant capacity strongly correlates with total polyphenol and flavonoid content ($R > 0.8$). Hydroxyferulic acid has been identified as the most abundant phenolic compound in amaranth leaves [11]. The trend in total flavonoid content in amaranth is similar to the trend in its antioxidant capacity, indicating that total flavonoids may be the main antioxidants in this vegetable [12].

Several previous studies have explored the use of spinach in cookie products with promising results. Research on red rice flour cookies with the addition of green spinach (*Amaranthus tricolor* L.) showed that there were significant differences in protein, fat, and carbohydrate content among various cookie formulations. The best formulation was obtained at a ratio of 90% red rice flour and 10% green spinach, with a protein content of 10.97%, fat 31%, carbohydrate 52.7%, and crude fiber 17.3% [13]. However, the addition of spinach in higher proportions tended to decrease sensory acceptability, especially in the aroma and taste aspects [13].

Despite growing interest in amaranth-based bakery products, most studies have focused on amaranth grain flour or green amaranth varieties, primarily evaluating macronutrient composition and physical properties. Limited research has simultaneously assessed iron content, antioxidant activity, and sensory acceptability of cookies substituted with red amaranth (*Amaranthus tricolor* L.) leaf flour a critical gap given that food-based fortification strategies for anemia prevention require products that are both nutritionally adequate and organoleptically acceptable to target populations. Furthermore, existing cookie fortification studies rarely quantify the contribution of iron per serving relative to the recommended dietary allowance, limiting their translational relevance for anemia intervention programs. Cookies, as widely consumed, shelf-stable snacks with established community distribution channels, represent a particularly suitable vehicle for iron fortification in low-resource settings. This study aimed to determine the optimal red amaranth flour substitution level in composite flour cookies that maximizes iron content and antioxidant activity while maintaining sensory acceptability, thereby informing the development of an affordable, iron-rich snack for anemia prevention.

2. METHOD

2.1. Design

This research method used a pure experimental design with a completely randomized design with one factor, namely the variation in the proportion of red spinach flour substitution to wheat flour in the cake formulation. There were five treatments determined based on the percentage of red spinach flour substitution, namely P0 (control, 0%), P1 (10%), P2 (20%), P3 (30%), and P4 (40%). Each treatment was repeated three times, so that the total experimental unit reached 15 replications, which allowed for adequate analysis of variance. The independent variable of this study was the percentage of red spinach flour substitution, while the dependent variables included Fe content, antioxidant activity (IC50 against DPPH), and sensory acceptance in the aspects of color, aroma, taste, texture, and overall acceptance. Controlled variables included the type and brand of raw materials other than red amaranth flour, baking temperature and time, cake size and thickness, and standard manufacturing procedures to maintain consistency between treatments.

2.2. Time and place of research

This study lasted for five months, from November 2025 to March 2026. Red amaranth flour was prepared at the Herbal Medicine Technology Laboratory, Department of Herbal Medicine, Poltekkes Kemenkes Surakarta, Indonesia, while iron content analysis using the AAS method was conducted at the UNS Analytical Chemistry Laboratory in Surakarta. Antioxidant activity analysis using the DPPH method was conducted at the Pharmacognosy and Phytochemistry Laboratory, Department of Herbal Medicine, while sensory testing was conducted in the sensory testing room of the Department of Herbal Medicine, which met lighting and ventilation standards. The work stages began with research preparation, including proposal preparation and ethical permitting, followed by red amaranth flour preparation and initial formulation testing. After that, cookies were made for all treatments with repetition, followed by laboratory analysis for Fe and DPPH, hedonic sensory testing, and finally data processing and analysis to compile the final report.

2.3. Material and procedure

The main research materials consisted of fresh red spinach of local varieties obtained from Pasar Gede Surakarta, low-protein wheat flour (certain brands), margarine, refined sugar, egg yolks, powdered milk, baking powder, vanilla powder, and salt. The red spinach used was the leaf part along with the young, soft stems. The chemicals needed for Fe analysis included concentrated HNO3, HClO4, Fe standard solution, and distilled water, while the materials for antioxidant analysis included DPPH, methanol for analysis, and ascorbic acid as a control. The tools needed included equipment for spinach processing (knife, blender, cabinet dryer, and 80 mesh sieve), cookie making equipment (scales, mixer, rolling pin, molds, baking pans, and oven), analytical equipment (AAS for Fe, muffle furnace, cuvette, UV-Vis for DPPH), and tools for sensory testing (three-digit code plate, score form, and sensory chamber).

The research procedure began with the production of red spinach flour by sorting the leaves and stems of tumba, washing, and blanching at a temperature of 90–95 °C for 3 minutes for enzyme inactivation and oxalate reduction, followed by rapid cooling in ice water. The blanching process is a common pretreatment applied to leafy vegetables at a temperature range of 70–100 °C for 1–15 minutes to stop enzymatic activity, maintain color, and reduce antinutrients such as oxalate [14]. High temperature blanching for a short time is more effective in preserving ascorbic acid than low-temperature treatment for a long duration [15]. As a quality control measure, the temperature and time of whitening are strictly controlled: a quick dip is applied for good whitening; for whitening (50 ± 2 °C) between 18 and 24 hours, for minimum thermal degradation, and for a whitening of life. Light to prevent oxidation during storage.

2.4. Formulation

Table 1 presents five cookie formulations with different levels of red spinach flour substitution (0%, 10%, 20%, 30%, and 40%) replacing wheat flour. The amounts of margarine, powdered sugar, egg yolk, milk powder, baking powder, vanilla powder, and salt were kept constant across all formulations. Therefore, the percentage of red spinach flour substitution was the only variable that differed among the formulations.

Iron content analysis was performed by wet digestion of the sample using a mixture of nitric acid and perchloric acid, gradually, until the solution was clear, then measured with AAS at a wavelength of 248.3 nm. The Fe standard curve was prepared from a 1000 ppm stock solution with several concentration points to ensure a high coefficient of determination. The analysis was performed in duplicate for each experimental unit to improve the accuracy of Fe measurements. Antioxidant activity analysis was performed by sample extraction using methanol maceration, solvent evaporation, and dilution of the extract solution to a concentration series for IC50 analysis through the percentage of DPPH radical inhibition at 517 nm. The test was performed in duplicate for each experimental unit.

Table 1. Cookie formulation with variations of red spinach flour substitution

Material	P0 (0%)	P1 (10%)	P2 (20%)	P3 (30%)	P4 (40%)
Wheat flour (g)	200	180	160	140	120
Red spinach flour (g)	0	20	40	60	80
Margarine (g)	120	120	120	120	120
Powdered sugar (g)	80	80	80	80	80
Egg yolk (piece)	2	2	2	2	2
Milk powder (g)	20	20	20	20	20
Baking powder (g)	2	2	2	2	2
Vanilla powder (g)	1	1	1	1	1
Salt (g)	1	1	1	1	1

2.5. Sensory test

Sensory testing used a panel of 30 semi-trained students from the Herbal Medicine Department of the Ministry of Health Polytechnic of Surakarta. Panelists assessed five sensory parameters—color, aroma, taste, texture, and overall acceptability using a five-point hedonic scale. Assessments were conducted in a completely randomized design with samples randomly presented on three-digit coded plates to avoid bias. Assessors were also provided with mineral water and plain crackers to neutralize the taste between samples. Hedonic data were analyzed using the non-parametric Kruskal-Wallis test due to the ordinal nature of the data, with a further Mann-Whitney test for pairwise comparisons if significant differences were found; data visualization was also provided in the form of bar charts or radar charts.

2.6. Sample size justification

The use of five treatments with three replications yielding 15 experimental units is consistent with the minimum requirement for a completely randomized design (CRD) in food science research. According to Federer's rule, the minimum number of replications satisfies the inequality $(t-1)\ (r-1) \geq 12$, where t is the number of treatments and r is the number of replications. With $t = 5$ and $r = 3$, the error degrees of freedom equal 10, which, while below the ideal threshold of 12, remains acceptable for preliminary experimental studies with laboratory-based analytical measurements conducted in duplicate. The 30 semi-trained panelists for sensory evaluation meet the minimum recommended panel size for hedonic testing.

2.7. Randomization process and production variability control

Treatment allocation to experimental units followed a CRD, wherein each of the 15 batches was randomly assigned a treatment using a random number generator. Sensory samples were presented in randomized order with three-digit codes to minimize order and expectation bias. Consistency was maintained by standardizing all ingredients (brand, batch), using identical baking temperature and duration, uniform dough weight and mold dimensions, and following a single standard operating procedure executed by one trained operator throughout all production runs.

2.8. Data processing

Data processing used SPSS version 26.0. Prerequisite tests included the Shapiro-Wilk normality test and Levene's homogeneity of variance test. For Fe and IC50 data, One-Way ANOVA analysis was performed if the assumptions were met, followed by Duncan's Multiple Range Test (DMRT) or Tukey honestly significant difference (HSD) as a post hoc test. If the assumptions were not met, Kruskal-Wallis non-parametric analysis followed by Mann-Whitney with Bonferroni correction. Correlation analysis between the proportion of red spinach substitution with Fe and IC50 used Pearson or Spearman according to the character of the data. All results are presented as mean ± SD, and significance was set at $\alpha = 0.05$.

2.9. Research flow

Figure 1 describes the sequence of activities, from red spinach procurement to data analysis and report preparation. The process begins with spinach raw material preparation, sorting, washing, blanching, drying, milling, and storage of red spinach flour. Next, cookies were formulated with various substitutions, cookies were made for each treatment, and samples were divided for Fe, DPPH, and sensory analysis. Data from the Fe, IC50, and sensory analysis were then statistically analyzed to provide an understanding of the relationship between red spinach substitutions and the tested properties, so that they can be evaluated as an iron-rich snack.

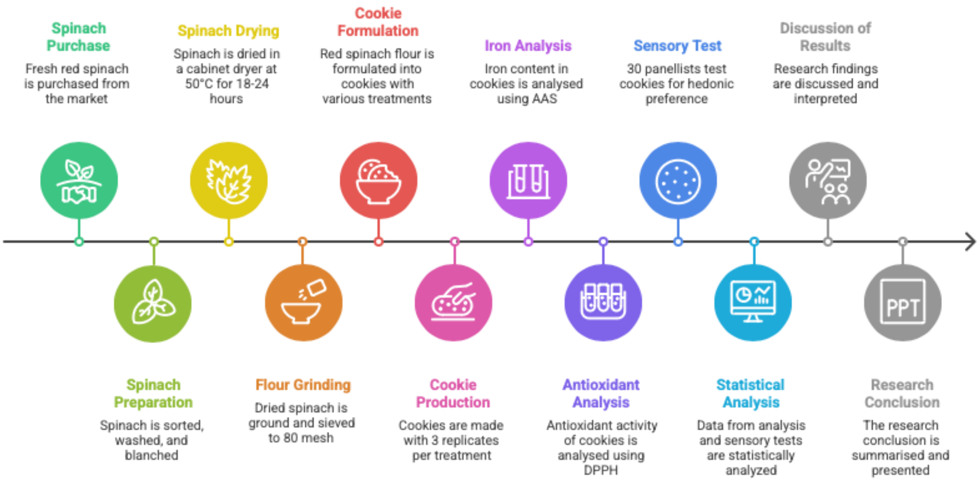

Figure 1. Research flow

3. RESULTS

Table 2 showed that substitution of red amaranth flour (*Amaranthus tricolor* L.) significantly increased the Fe content of cookies proportionally to the substitution level (p<0.05). The Fe content of control cookies (P0.0%) of 2.14 ± 0.12 mg/100 g increased gradually to 9.31 ± 0.34 mg/100 g at 40% substitution (P4), which is equivalent to an increase of 335.05% compared to the control. Each treatment showed significant differences from each other based on the DMRT test, indicating a dose-dependent relationship between the proportion of red amaranth flour and the Fe content of cookies. This increase is in line with the high iron content in red amaranth which is known to contain Fe around 2.2–7.0 mg/100 g fresh weight, so that its substitution directly contributes to the natural fortification of cookie products.

Table 3 demonstrates that red amaranth (*Amaranthus tricolor* L.) flour substitution significantly increased cookie antioxidant activity based on the DPPH assay ($p<0.05$). The IC_{50} value decreased progressively from 487.32 ± 18.45 µg/mL (control, very weak) to 112.37 ± 7.24 µg/mL (40% substitution, moderate) per Molyneux's (2004) classification, attributed to bioactive compounds such as betacyanins, flavonoids, and phenolic compounds. Tukey HSD confirmed significant differences among all treatments. However, all formulations remained substantially lower than the ascorbic acid positive control (IC_{50} = 4.57 ± 0.31 µg/mL).

Table 4 shows that increasing red amaranth flour substitution significantly decreased sensory acceptability scores across all attributes ($p < 0.001$), with formulations P0 (0%) and P1 (10%) consistently not significantly different and achieving the highest scores (range 3.87–4.17, “like” category), while P4 (40%) achieved the lowest scores across all attributes (2.67–3.13, “neutral” to “dislike” category). Texture attributes demonstrated the highest supply resilience to substitution, with significant differences emerging only in P4. In the context of public health nutrition, these findings hold important reminders given the persistently high prevalence of iron deficiency anemia and micronutrient deficiencies in Indonesia, particularly among children and women of childbearing age. Red amaranth (*Amaranthus tricolor* L.) is known as an affordable and readily locally cultivated source of iron, beta-carotene, and antioxidants, making fortification of street foods such as cookies a potential strategic food-based approach to addressing hidden hunger.

Table 2. Iron content of cookies with varying levels of red spinach flour substitution

Treatment	Red spinach flour substitution (%)	Iron content (mg/100 g)	Increase relative to control (%)
P0	0	2.14 ± 0.12^{a}	—
P1	10	3.87 ± 0.15^{b}	80.84
P2	20	5.62 ± 0.21^{c}	162.62
P3	30	7.49 ± 0.28^{d}	250.00
P4	40	9.31 ± 0.34^{e}	335.05

Notes: Values are expressed as mean ± standard deviation (n = 3, each measured in duplicate). Means followed by different superscript letters within the same column are significantly different (One-Way ANOVA followed by DMRT, $p<0.05$). The iron content was determined by Atomic Absorption Spectrophotometry (AAS) at a wavelength of 248.3 nm following wet acid digestion ($HNO_3/HClO_4$). The coefficient of determination (R^2) of the standard calibration curve was ≥0.999.

Table 3. Antioxidant activity (IC_{50} DPPH) of cookies with varying levels of red spinach flour substitution

Treatment	Red spinach flour substitution (%)	IC_{50} (µg/mL)	Antioxidant strength category
P0	0	487.32 ± 18.45^{a}	Very weak
P1	10	362.18 ± 14.73^{b}	Weak
P2	20	258.41 ± 11.56^{c}	Weak
P3	30	174.65 ± 9.82^{d}	Moderate
P4	40	112.37 ± 7.24^{e}	Moderate

Notes: Values are expressed as mean ± standard deviation (n = 3, each measured in duplicate). Means followed by different superscript letters within the same column are significantly different (One-Way ANOVA followed by Tukey HSD, $p<0.05$). IC_{50} represents the concentration of sample extract required to inhibit 50% of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals, measured spectrophotometrically at 517 nm. A lower IC_{50} value indicates stronger antioxidant activity. Antioxidant strength categories follow the classification of Molyneux (2004): very strong (IC_{50} <50 µg/mL), strong (50–100 µg/mL), moderate (100–250 µg/mL), weak (250–500 µg/mL), and very weak (IC_{50} > 500 µg/mL). Ascorbic acid was used as a positive control (IC_{50} = 4.57 ± 0.31 µg/mL).

Table 4. Sensory acceptability scores (hedonic test) of cookies with varying levels of red spinach flour substitution

Sensory attribute	P0 (0%)	P1 (10%)	P2 (20%)	P3 (30%)	P4 (40%)	p-value (Kruskal-Wallis)
Color	4.17 ± 0.65^{a}	3.90 ± 0.71^{ab}	3.63 ± 0.72^{b}	3.13 ± 0.78^{c}	2.73 ± 0.83^{d}	< 0.001*
Aroma	4.03 ± 0.61^{a}	3.87 ± 0.63^{a}	3.60 ± 0.67^{ab}	3.27 ± 0.74^{b}	2.90 ± 0.80^{c}	< 0.001*
Taste	4.10 ± 0.55^{a}	3.93 ± 0.58^{a}	3.67 ± 0.66^{ab}	3.17 ± 0.75^{b}	2.67 ± 0.84^{c}	< 0.001*
Texture	4.07 ± 0.58^{a}	3.97 ± 0.61^{a}	3.77 ± 0.63^{a}	3.47 ± 0.68^{ab}	3.13 ± 0.73^{b}	0.001*
Overall acceptability	4.13 ± 0.51^{a}	3.93 ± 0.52^{a}	3.67 ± 0.61^{ab}	3.20 ± 0.71^{b}	2.80 ± 0.76^{c}	< 0.001*

Notes: Values are expressed as median-referenced mean ± standard deviation (n = 30 panelists). Hedonic scale: 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, 5 = strongly like. Means followed by different superscript letters within the same row are significantly different based on pairwise Mann-Whitney U tests with Bonferroni correction ($p<0.05$). The asterisk (*) denotes a statistically significant Kruskal-Wallis test result at α = 0.05. Panelists were 30 semi-trained students from the Department of Jamu, Poltekkes Kemenkes Surakarta. Samples were presented in randomized order on plates coded with three-digit random numbers.

Table 5 demonstrates that increasing red spinach flour concentration linearly enhanced iron content (2.14–9.31 mg/100 g) and antioxidant activity (IC_{50} decreasing from 487.32 to 112.37 µg/mL), yet inversely affected sensory acceptability, with scores declining significantly from 4.13 (P0) to 2.80 (P4). Treatment P2 (20%) emerged as the optimal formulation, providing 9.37% of the Indonesian RDA for iron per 30 g serving while maintaining acceptable sensory scores, positioning it as a viable food-based strategy to address iron deficiency anemia—a persistent public health concern affecting approximately 27.2% of Indonesian women of reproductive age (Ministry of Health, 2018). However, given the relatively modest iron contribution per serving (<16% RDA), these cookies should be contextualized as a complementary dietary vehicle rather than a primary intervention, aligning with WHO recommendations for sustainable, food-based approaches to combat micronutrient deficiencies through locally available ingredients.

Table 5. Summary of iron content, antioxidant activity, and overall sensory acceptability across treatments with evaluation against nutritional benchmarks

Treatment	Red spinach flour (%)	Iron content (mg/100 g)	% RDA Iron per Serving (30 g)*	IC_{50} DPPH (µg/mL)	Overall acceptability score	Acceptability category
P0	0	2.14 ± 0.12^{a}	3.57	487.32 ± 18.45^{a}	4.13 ± 0.51^{a}	Liked
P1	10	3.87 ± 0.15^{b}	6.45	362.18 ± 14.73^{b}	3.93 ± 0.52^{a}	Liked
P2	20	5.62 ± 0.2^{c}	9.37	258.41 ± 11.56^{c}	3.67 ± 0.61^{ab}	Neutral–liked
P3	30	7.49 ± 0.28^{d}	12.49	174.65 ± 9.82^{d}	3.20 ± 0.71^{b}	Neutral
P4	40	9.31 ± 0.34^{e}	15.52	112.37 ± 7.24^{e}	2.80 ± 0.76^{c}	Neutral–disliked

Notes: Values are expressed as mean ± standard deviation. Means followed by different superscript letters within the same column are significantly different ($p< 0.05$). *The percentage of Recommended Dietary Allowance (%RDA) for iron is calculated based on a single serving size of 30 g (approximately 3–4 cookies) relative to the Indonesian RDA for iron for adult women aged 19–29 years (18 mg/day) as specified by the Regulation of the Minister of Health of the Republic of Indonesia Number 28 of 2019 concerning the Recommended Dietary Allowance for Indonesians (Nutrition Adequacy Figures/AKG). Acceptability categories are interpreted as follows: score ≥ 3.80 = "Liked"; 3.40–3.79 = "Neutral–Liked"; 3.00–3.39 = "Neutral"; < 3.00 = "Neutral–Disliked."

4. DISCUSSION

Substitution of red amaranth (*Amaranthus tricolor* L.) flour significantly increased cookie iron content ($p < 0.05$). Control cookies contained 2.14 mg Fe/100 g, while 40% substitution yielded 9.31 mg Fe/100 g approximately 4.35 times higher. Amaranth is recognized as an iron-rich plant essential for red blood cell formation [16]. Amaranth seeds also contain high levels of iron (72–174 ppm) [17], and its flour has a mineral content 2–3 times higher than that of wheat [18]. This pattern of progressively increasing iron levels with increasing substitution proportions is consistent with findings in other bakery products. Amaranth flour substitution in chapatti up to 40% significantly increased the iron content [19]. Fortification of amaranth and *Lupinus* mutabilis flour-based products showed a marked increase in iron compounds with direct implications in combating anemia [20]. A very strong Pearson correlation between the percentage of red amaranth flour substitution and iron content ($r = 0.998$, $p < 0.001$) confirmed a nearly linear dose-response relationship. This linearity indicates that the iron contributed by red amaranth flour was largely retained during the baking process, with minimal thermal degradation of the mineral [21].

At 20% substitution (P2), a 30 g cookie serving provides 9.37% of the iron RDI for adult women, while 40% substitution (P4) reaches 15.52%, nearly meeting Indonesian "good source of iron" labeling criteria. The antioxidant activity of cookies increased significantly with increasing red amaranth flour substitution, as evidenced by a progressive decrease in DPPH IC_{50} values ($p < 0.05$). Control cookies (P0) showed very weak antioxidant activity ($IC_{50} = 487.32 \pm 18.45$ µg/mL), while at 40% substitution (P4), the IC_{50} decreased to 112.37 ± 7.24 µg/mL, placing the cookies in the moderate antioxidant activity category classification. This 76.94% decrease in IC_{50} indicates a substantial increase in free radical scavenging capacity.

The increased antioxidant activity reflects the rich phytochemical profile of *Amaranthus tricolor* L. Baraniak and Kania-Dobrowolska [22] noted that the *Amaranthus* genus contains phenolic compounds, flavonoids, betalains, and tocopherols with significant antioxidant activity, varying by developmental stage as measured through $ABTS^{+}$, DPPH, FRAP, and Fe^{2+} chelation methods. A significant increase in DPPH radical scavenging activity in cookies with the addition of bok choy puree, and noted that phenolic compounds in wheat flour also contributed to the basal antioxidant activity in control cookies, which is consistent with the non-zero IC_{50} value of control cookies in this study [23].

Similar findings have been reported in various studies of fortification of bakery products with antioxidant-rich plant ingredients. Substitution of wheat flour with jackfruit seed flour increased total polyphenol content and DPPH radical scavenging activity progressively with increasing substitution ratio [24]. Cookies fortified with Zizyphus lotus powder exhibited higher free radical scavenging and reducing power activity than control cookies, with activity increasing with increasing substitution level [25]. A strong negative Spearman correlation between the percentage of substitution and IC_{50} values ($r_s = -0.993$, $p < 0.001$) confirmed that higher levels of substitution were consistently associated with stronger antioxidant activity. However, it should be noted that the thermal process during baking (150 °C, 20–25 min) may have degraded some heat-labile antioxidants such as ascorbic acid and certain betalain pigments. A significant decrease in β-carotene levels occurred in stored cookies due to non-oxidative changes (cis-trans isomerization, epoxide formation) and oxidative changes [26].

For the color attribute, the decrease in score from 4.17 (P0, "like") to 2.73 (P4, between "dislike" and "neutral") was due to the progressive darkening of the cookie color from golden brown to dark greenish brown. This color change was attributed to chlorophyll, betalain pigments, and Maillard reaction products formed between amino acids in red amaranth flour and reducing sugars during baking [27]. The addition of green amaranth (*Amaranthus tricolor* L.) flour to nuggets resulted in a darker color as the percentage increased, as the chlorophyll in amaranth provides a darker color effect on the food product [28].

Aroma and taste scores were statistically comparable between P0 and P1 ($p > 0.05$), indicating no detectable off-flavors at 10% substitution. At higher substitution levels (30–40%), grassy aroma and bitterness from oxalates, saponins, and tannins became more pronounced, consistent with findings reported by Riaz and Wahab [29]. Van *et al.* [30] also reported that sensory scores for taste, color, and aroma tended to decrease with increasing substitution ratios of jackfruit seed flour, with the highest overall acceptability scores at 10% substitution and the lowest at 40%.

Integration of the three main outcome variables (iron content, antioxidant activity, and sensory acceptability) indicated that formulation P2 (20% substitution of red amaranth flour) was the most promising candidate for further development. This formulation achieved a 162.62% increase in iron content and a 46.98% decrease in IC_{50} compared to the control, while maintaining an overall acceptability score (3.67 ± 0.61) that was not significantly different from the control ($p > 0.05$). These findings are consistent with various studies that recommend a substitution level of 15–20% as optimal for bakery products fortified with vegetable flours [31], [32].

5. CONCLUSION

The substitution of red amaranth (*Amaranthus tricolor* L.) flour in cookies significantly increased iron content and antioxidant activity in a dose-dependent manner ($p < 0.05$), yet was inversely proportional to sensory acceptability. Considering the balance between nutritional value and acceptability, the 20% substitution level (P2) was identified as the most optimal formulation, yielding a meaningful increase in iron content while maintaining sensory acceptability that was not significantly different from the control. These findings suggest that red amaranth flour-based cookies hold potential as a functional food in the prevention of iron deficiency anemia, particularly as a locally sourced, food-based fortification strategy that is both practical and acceptable for community-level intervention. Further research is warranted to enhance iron bioavailability and improve sensory characteristics at higher substitution levels, thereby optimizing the product's contribution to dietary iron adequacy in at-risk populations.

FUNDING INFORMATION

This research was funded by the Ministry of Health through the funding system at the Poltekkes Kemenkes Surakarta with number 112/Poltekkes Surakarta/2025.

AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Yeni Tutu Rohimah	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dwi Retna Prihati	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Titik Lestari	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT
Authors state no conflict of interest.

ETHICAL APPROVAL
Poltekkes Kemenkes Surakarta with the number: Etik/LH/2207/Poltekkes Kemenkes Surakarta/2025.

DATA AVAILABILITY
The data that support the findings of this study are available from the corresponding author, [YTR], upon reasonable request.

REFERENCES
[1] Amila and E. Sembiring, "THE effect of beetroot biscuits (Beta vulgaris) on the hemoglobin level of patients with pulmonary tuberculosis," *Indonesian Journal of Public Health*, vol. 16, no. 1, pp. 43–55, Apr. 2021, doi: 10.20473/ijph.v16i1.2021.43-55.
[2] A. Bechoff, J. de Bruyn, A. Alpha, F. Wieringa, and V. Greffeuille, "Exploring the complementarity of fortification and dietary diversification to combat micronutrient deficiencies: a scoping review," *Current Developments in Nutrition*, vol. 7, no. 2, pp. 1–19, Feb. 2023, doi: 10.1016/j.cdnut.2023.100033.
[3] B. Kolarš *et al.*, "Iron deficiency and iron deficiency anemia: a comprehensive overview of established and emerging concepts," *Pharmaceuticals*, vol. 18, no. 8, pp. 1–47, Jul. 2025, doi: 10.3390/ph18081104.
[4] P. Sari, R. T. D. Judistiani, D. Hilmanto, D. M. D. Herawati, and M. Dhamayanti, "Iron deficiency anemia and associated factors among adolescent girls and women in a rural area of Jatinangor, Indonesia," *International Journal of Women's Health*, vol. 14, no. 8, pp. 1137–1147, Aug. 2022, doi: 10.2147/IJWH.S376023.
[5] U. Sarker, Y. P. Lin, S. Oba, Y. Yoshioka, and K. Hoshikawa, "Prospects and potentials of underutilized leafy amaranths as vegetable use for health-promotion," *Plant Physiology and Biochemistry*, vol. 182, no. 7, pp. 104–123, Jul. 2022, doi: 10.1016/j.plaphy.2022.04.011.
[6] R. N. Olusanya, U. Kolanisi, and N. Z. Ngobese, "Mineral composition and consumer acceptability of amaranthus leaf powder supplemented ujeqe for improved nutrition security," *Foods*, vol. 12, no. 11, pp. 1–17, May 2023, doi: 10.3390/foods12112182.
[7] X. Zhao, M. Zhu, X. Ren, Q. An, J. Sun, and D. Zhu, "A new technique for determining micronutrient nutritional quality in fruits and vegetables based on the entropy weight method and fuzzy recognition method," *Foods*, vol. 11, no. 23, pp. 1–14, Nov. 2022, doi: 10.3390/foods11233844.
[8] M. T. Yilma, A. Eifa, M. Belayneh, and A. Z. Orsango, "Effect of amaranth-containing dietary intervention in improving hemoglobin concentration: a systematic review and meta-analysis," *Public Health Reviews*, vol. 45, no. 1, pp. 1–10, Jan. 2024, doi: 10.3389/phrs.2024.1607597.
[9] T. S. Johnson, A. de'Souza, S. A. Patil, and A. Vora, "Safety assessment of red spinach as a potential sports nutrition supplement: an evidence based comprehensive overview," *Future Foods*, vol. 12, no. 12, pp. 1–11, Dec. 2025, doi: 10.1016/j.fufo.2025.100806.
[10] K. VN, V. Rajasree, R. Swarnapriya, D. Uma, and P. Meenakshi, "Nutrient availability of selected leafy vegetables at micro green stage grown in vertical gardening," *Journal of Pharmacognosy and Phytochemistry*, vol. 10, no. 1, pp. 2226–2228, Jan. 2021, doi: 10.22271/phyto.2021.v10.i1ae.13686.
[11] U. Sarker, M. M. Hossain, and S. Oba, "Nutritional and antioxidant components and antioxidant capacity in green morph amaranthus leafy vegetable," *Scientific Reports*, vol. 10, no. 1, pp. 1–10, Jan. 2020, doi: 10.1038/s41598-020-57687-3.
[12] Y. Zhang *et al.*, "Variation in the main health-promoting compounds and antioxidant capacity of three leafy vegetables in southwest China," *Molecules*, vol. 28, no. 12, pp. 1–17, Jun. 2023, doi: 10.3390/molecules28124780.
[13] N. A. Mahmudah, R. N. Mauizza, H. Nabawiyah, and A. Y. Damayanti, "Nutritional value and sensory properties of brown rice flour cookies with green spinach (*Amaranthus tricolor* L.) incorporation as gluten-free food alternative," *Jurnal Teknologi Pangan dan Hasil Pertanian*, vol. 18, no. 1, pp. 33–40, Feb. 2023, doi: 10.26623/jtphp.v18i1.6110.
[14] B. M. Mugo, J. Kiio, and A. Munyaka, "Effect of blanching time–temperature on potassium and vitamin retention/loss in kale and spinach," *Food Science and Nutrition*, vol. 12, no. 8, pp. 5403–5411, Aug. 2024, doi: 10.1002/fsn3.4186.
[15] M. Mieszczakowska-Frąc, K. Celejewska, and W. Płocharski, "Impact of innovative technologies on the content of vitamin c and its bioavailability from processed fruit and vegetable products," *Antioxidants*, vol. 10, no. 1, pp. 1–19, Jan. 2021, doi: 10.3390/antiox10010054.
[16] A. Z. Orsango, E. Loha, B. Lindtjørn, and I. M. S. Engebretsen, "Efficacy of processed amaranth-containing bread compared to maize bread on hemoglobin, anemia and iron deficiency anemia prevalence among two-to-five year-old anemic children in southern Ethiopia: a cluster randomized controlled trial," *PLoS ONE*, vol. 15, no. 9 September, pp. 1–16, Sep. 2020, doi: 10.1371/journal.pone.0239192.
[17] D. Toimbayeva *et al.*, "Prospects for the use of amaranth grain in the production of functional and specialized food products," *Foods*, vol. 14, no. 9, pp. 1–36, May 2025, doi: 10.3390/foods14091603.
[18] H. Kumar *et al.*, "Amaranth and buckwheat grains: nutritional profile, development of functional foods, their pre-clinical cum clinical aspects and enrichment in feed," *Current Research in Food Science*, vol. 9, no. 9, pp. 1–19, 2024, doi: 10.1016/j.crfs.2024.100836.
[19] A. Banerji, L. Ananthanarayan, and S. Lele, "Rheological and nutritional studies of amaranth enriched wheat chapatti (Indian flat bread)," *Journal of Food Processing and Preservation*, vol. 42, no. 1, pp. 1–12, Jan. 2018, doi: 10.1111/jfpp.13361.
[20] O. M. Quelal and M. B. Pulles, "Nutritional, functional and microbiological potential of Andean lupinus mutabilis and amaranthus spp. in the development of healthy foods—a review," *Foods*, vol. 14, no. 12, pp. 1–23, Jun. 2025, doi: 10.3390/foods14122059.
[21] I. Coțovanu, S. G. Stroe, F. Ursachi, and S. Mironeasa, "Addition of amaranth flour of different particle sizes at established doses in wheat flour to achieve a nutritional improved wheat bread," *Foods*, vol. 12, no. 1, pp. 1–18, 2023, doi: 10.3390/foods12010133.

[22] J. Baraniak and M. Kania-Dobrowolska, "The dual nature of amaranth—functional food and potential medicine," *Foods*, vol. 11, no. 4, pp. 1–12, Feb. 2022, doi: 10.3390/foods11040618.
[23] S. Baliyan *et al.*, "Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of ficus religiosa," *Molecules*, vol. 27, no. 4, pp. 1–19, Feb. 2022, doi: 10.3390/molecules27041326.
[24] C. M. Nguyen *et al.*, "Low-glycemic index cookies supplemented with cordyceps militaris substrate: nutritional values, physicochemical properties, antioxidant activity, bioactive constituents, and bioaccessibility," *Food Chemistry: X*, vol. 27, no. 4, pp. 1–9, Apr. 2025, doi: 10.1016/j.fochx.2025.102494.
[25] A. Krajewska and D. Dziki, "Enrichment of cookies with fruits and their by-products: chemical composition, antioxidant properties, and sensory changes," *Molecules*, vol. 28, no. 10, pp. 1–26, May 2023, doi: 10.3390/molecules28104005.
[26] H. M. Ali, S. A. Eid, H. A. S. Eldeen, and M. E. H. Ebrahim, "Oxidative degradation pathways of carrot carotenes and their protection via chitosan-tpp encapsulation," *Chemical and Biological Technologies in Agriculture*, vol. 12, no. 1, pp. 1–16, Sep. 2025, doi: 10.1186/s40538-025-00855-2.
[27] L. R. Chamidah and D. Amertaningtyas, "The effect of adding green spinach flour (*amaranthus tricolor*) on chicken liver nuggets in terms of yield quality, organoleptic preference, and color using a color reader," in *ICESAI 2022*, 2023, pp. 185–194, doi: 10.2991/978-94-6463-116-6_24.
[28] S. Arunachalam, B. Rajoo, H. K. Ravi, and S. Murugesan, "Optimization of nutrient-enriched ravioli incorporating elephant foot yam flour and encapsulated okra–moringa pearls," *Applied Sciences (Switzerland)*, vol. 16, no. 5, pp. 1–32, 2026, doi: 10.3390/app16052435.
[29] A. Riaz and S. Wahab, "Efficacy of moringa leaves powder as a supplement in whole wheat flour biscuit," *Sarhad Journal of Agriculture*, vol. 37, no. 2, pp. 573–585, 2021, doi: 10.17582/JOURNAL.SJA/2021/37.2.573.585.
[30] C. K. Van, T. H. Nguyen, T. T. N. H. Nguyen, P. T. N. Nguyen, T. T. Tran, and Q. B. Hoang, "Comparison of the effects of jackfruit seed flour and jackfruit seed starch in the cookie manufacturing process," *Processes*, vol. 11, no. 11, pp. 1–17, Nov. 2023, doi: 10.3390/pr11113194.
[31] V. Imeneo, F. Tokatli, B. Ozen, C. Cappa, I. Aguiló-Aguayo, and C. Alamprese, "Incorporating legume and nut flours into pasta, bakery products, and snacks: opportunities and challenges," *Future Foods*, vol. 12, no. 12, pp. 1–25, 2025, doi: 10.1016/j.fufo.2025.100811.
[32] X. Xie, K. Cai, Z. Yuan, L. Shang, and L. Deng, "Effect of mealworm powder substitution on the properties of high-gluten wheat dough and bread based on different baking methods," *Foods*, vol. 11, no. 24, 2022, doi: 10.3390/foods11244057.

BIOGRAPHIES OF AUTHORS

Yeni Tutu Rohimah is an academic faculty member in the Department of Nursing at Poltekkes Kemenkes Surakarta, Surakarta, Indonesia. She is affiliated with one of Indonesia's prominent health polytechnics operating under the auspices of the Ministry of Health of the Republic of Indonesia, an institution dedicated to producing competent healthcare professionals, particularly in the field of nursing. Her academic and professional activities are centered on nursing education and practice, contributing to the development of nursing science and the advancement of healthcare workforce training in Central Java, Indonesia. Through her role at Poltekkes Kemenkes Surakarta, she is engaged in teaching, research, and community service in alignment with the Tri Dharma Perguruan Tinggi framework in Indonesia. She can be contacted at email: yenitutur@gmail.com.

Dwi Retna Prihati is an academic faculty member affiliated with the Department of Midwifery, Poltekkes Kemenkes Surakarta, Surakarta, Indonesia. She is engaged in teaching and research within the field of midwifery, contributing to the education and professional development of midwifery practitioners in Indonesia. Her academic work is situated within the context of maternal and reproductive health, aligning with the institutional mission of Poltekkes Kemenkes Surakarta to produce competent health professionals. As a member of a government-affiliated health polytechnic under the Indonesian Ministry of Health (*Kementerian Kesehatan Republik Indonesia*), her professional activities encompass both educational instruction and applied health research relevant to community health outcomes in Central Java and beyond. She can be contacted at email: dwiretna07@gmail.com.

Titik Lestari is an academic faculty member in the Department of Nursing at Poltekkes Kemenkes Surakarta, Surakarta, Indonesia. She is affiliated with one of the prominent health polytechnics under the Indonesian Ministry of Health, an institution dedicated to producing competent healthcare professionals, particularly in the field of nursing. Her academic and professional activities are centered on nursing education and practice within the Indonesian healthcare system. As a lecturer at Poltekkes Kemenkes Surakarta, she contributes to the development of nursing science through teaching, research, and community service, in alignment with the Tri Dharma Perguruan Tinggi (Three Pillars of Higher Education) framework in Indonesia. Her research interests encompass areas within nursing science and healthcare. She can be contacted at email: titidwilest@gmail.com.