

# Effect of *Moringa oleifera* Leaf Flour Addition on Iron (Fe) and Vitamin A Levels of Crackers as a Healthy Snack

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## Abstract

*Moringa (Moringa oleifera)* leaf crackers are an innovative food product that can be utilized as a nutritious snack. *Moringa* is widely recognized as a superfood due to its high nutritional value, including proteins, vitamins, and minerals that provide various health benefits. In the context of improving community dietary patterns, these crackers may serve as a healthier snack alternative. This study aimed to evaluate the effectiveness of *Moringa oleifera* leaf supplementation in increasing the iron (Fe) and vitamin A contents of crackers as a healthy snack alternative. The analyses were conducted using a spectrophotometric method with standard calibration curves to determine iron and vitamin A concentrations. The treatments consisted of different concentrations of *Moringa* leaf powder: 0%, 5%, 10%, and 15%. The results showed that the addition of *Moringa* leaf powder significantly increased the iron content, from 0.67 mg/100 g in the control treatment to 3.05 mg/100 g at the highest concentration. Vitamin A content also increased with increasing levels of *Moringa* leaf supplementation, although the trend was not entirely linear due to the effects of processing on compound stability. The standard calibration curves demonstrated a linear relationship between concentration and absorbance, indicating that the analytical method was valid. These findings suggest that the incorporation of *Moringa oleifera* leaf powder effectively enhances the nutritional quality of crackers, particularly in terms of iron and vitamin A content, making them a promising healthy snack option.

**Keywords:** *Moringa oleifera*; crackers; iron; vitamin A; healthy snack; spectrophotometry.

## INTRODUCTION

Indonesia is rich in plant biodiversity that provides numerous health benefits and contains abundant nutrients, both macronutrients and micronutrients. One of the plants widely utilized as both a food source and a medicinal ingredient is *Moringa oleifera* L. Commonly known as moringa, this plant belongs to the family Moringaceae and is referred to by various local names across different regions of Indonesia, including kelor, kerol, marangghi, moltong, kelo, kelo, kawano, and ongge. *Moringa oleifera* can grow well in both lowland and highland areas, reaching a height of approximately 7–11 meters under favorable environmental conditions (Darna et al., 2019). The incorporation of moringa leaves into food products has attracted considerable attention because of their potential to enhance nutritional quality, particularly the levels of iron and vitamin A. *Moringa* leaves contain a wide range of essential minerals and vitamins that contribute significantly to human health. Iron plays a crucial role in hemoglobin synthesis and helps prevent iron-deficiency anemia, while vitamin A is essential for maintaining eye health and strengthening the immune system. Increasing the proportion of moringa

leaf supplementation in food products generally results in higher concentrations of these nutrients, highlighting the potential of moringa leaves as a natural fortification ingredient for improving the nutritional value of foods. Previous studies have demonstrated that moringa leaves are rich sources of micronutrients, especially iron and vitamin A, which provide important health benefits (Kurniawan et al., 2020).

Iron (Fe) is an essential trace mineral required by the human body for the synthesis of hemoglobin in red blood cells. Hemoglobin plays a fundamental role in binding and transporting oxygen from the respiratory system to tissues throughout the body. In addition, iron contributes to energy metabolism, cell proliferation, and the maintenance of immune function. Iron deficiency can lead to anemia, characterized by symptoms such as excessive fatigue, reduced physical performance, and impaired concentration in both academic and daily activities (Putri & Handayani, 2022). *Moringa* leaves contain substantially higher iron levels than many other green leafy vegetables, making them an excellent source of dietary iron supplementation. The iron content of dried moringa leaves can reach approximately 28 mg per 100 g, indicating their strong potential for nutritional

enrichment of food products (Sadha, 2022). Consequently, the incorporation of moringa leaves into crackers may substantially improve their nutritional value. Adequate iron intake is necessary for red blood cell formation and various metabolic processes; therefore, dietary sources rich in iron are essential for maintaining optimal health (Novitaroh et al., 2022).

Vitamin A is a fat-soluble vitamin that plays a crucial role in maintaining overall health and supporting cell growth and development. In moringa leaves, vitamin A is present primarily in the form of  $\beta$ -carotene, which functions as a provitamin A compound. The high  $\beta$ -carotene content of moringa leaves makes this plant a promising ingredient for food fortification aimed at enhancing the nutritional quality of processed food products. Previous studies have reported that moringa leaves contain substantial amounts of vitamin A, contributing significantly to daily vitamin A requirements and improving the nutritional value of food products (Velayati et al., 2023). Vitamin A is an essential component of the retina and plays a vital role in visual function. Furthermore, it contributes to the maintenance of the immune system, regulates cellular differentiation, supports reproductive processes, and may help reduce the risk of certain cancers and cardiovascular diseases. Consequently, vitamin A serves both preventive and therapeutic functions against a variety of health disorders. Moringa leaves are recognized for their exceptional nutritional profile, particularly their vitamin A content, which is estimated to be approximately ten times higher than that of carrots. In addition, moringa leaves contain calcium levels seventeen times greater than milk, potassium levels fifteen times higher than bananas, protein levels nine times greater than yogurt, and iron concentrations twenty-five times higher than spinach (Fatmawati et al., 2020).

Crackers are among the most popular snack foods in Indonesia. Owing to their pleasant taste and crispy texture, crackers are commonly consumed either as accompaniments to main dishes or as everyday snacks. However, many commercially available crackers generally possess limited nutritional value, particularly with respect to iron and vitamin A content, both of which are essential for maintaining health, preventing anemia, and supporting visual function (Ramadani et al., 2020). *Moringa oleifera* leaf crackers represent an innovative food product that can be utilized as a nutritious snack. Moringa is widely recognized as a superfood due to its rich nutritional composition, including proteins, vitamins, and minerals that provide numerous health benefits. In the context of promoting healthier dietary habits among the population, moringa leaf crackers offer an attractive and nutritious snack alternative (Qadir et al., 2022). Therefore, this study aimed to evaluate the effectiveness of *Moringa oleifera* leaf supplementation in increasing the iron (Fe) and vitamin A contents of crackers as a healthy snack alternative.

## MATERIALS AND METHODS

### Study Area

This study was conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tadulako, in April 2026. The laboratory environment was well maintained, clean, and met the required standards for educational and research activities. The research employed an experimental design using a Completely Randomized Design (CRD) consisting of four treatment levels of *Moringa oleifera* leaf supplementation (0%, 5%, 10%, and 15%) with two replications for each treatment. The iron (Fe) and vitamin A contents of the cracker samples were analyzed using UV-Visible (UV-Vis) spectrophotometry.

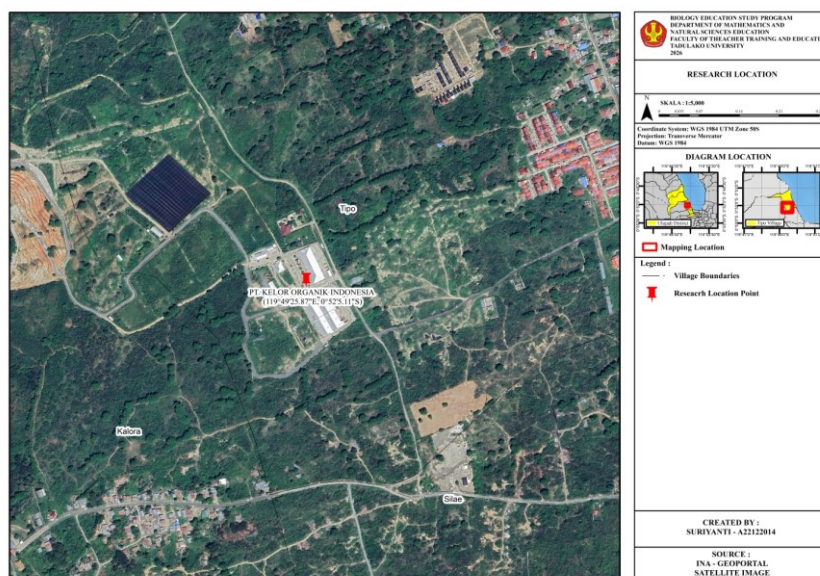


Figure 1. Map of the research location in PT. Kelor Organik Indonesia, Central Sulawesi, Indonesia.

## Procedures

### *Preparation of Materials and Equipment*

The primary samples used in this study were *Moringa oleifera* leaf crackers formulated with different concentrations of moringa leaf flour, namely 0%, 5%, 10%, and 15%. The cracker samples were first ground into a fine powder to ensure homogeneity. The powdered samples were then transferred into Erlenmeyer flasks and accurately weighed prior to analysis. The equipment used in this study included a mortar and pestle, analytical balance, Erlenmeyer flasks, funnels, Whatman filter paper, aluminum foil, beakers, fume hood, UV-Visible spectrophotometer, dropper pipettes, volumetric pipettes, test tubes, test tube racks, labeling materials, a digital camera, and stationery.

### *Sample Digestion Procedure*

The digestion procedure began by accurately weighing approximately 0.5–1.0 g of the powdered sample using an analytical balance, and the initial sample weight was recorded. The sample was then transferred into an Erlenmeyer flask, followed by the careful addition of an acid mixture consisting of 3 mL of concentrated nitric acid (HNO<sub>3</sub>) and 1 mL of hydrochloric acid (HCl). The mixture was heated on a hot plate at approximately 80–90°C for 1–3 hours until complete digestion was achieved and the solution became clear. After heating, the solution was allowed to cool to room temperature and subsequently filtered to remove any insoluble residues. The filtrate was then transferred into a volumetric flask and diluted with distilled water to a final volume of 50 mL.

### *Determination of Iron (Fe) Content Using UV-Vis Spectrophotometry*

5 mL of 1 M HCl solution was added to the digested sample solution. The mixture was transferred into a 10 mL volumetric flask and diluted to the calibration mark with distilled water. Subsequently, 2 mL of this solution was transferred into another 10 mL volumetric flask. Then, 1 mL of potassium thiocyanate (KSCN) solution and 1 mL of 4 N HCl solution were added, and the mixture was diluted to volume with distilled water. The absorbance of the resulting solution was measured at the predetermined maximum wavelength using a UV-Visible spectrophotometer. The iron concentration in the sample was calculated using the regression equation obtained from the Fe standard calibration curve ( $y = ax + b$ ). The iron content was determined using the following equation:

$$\text{Iron (Fe) (mg/100g)} = \frac{x \cdot Y \cdot fp}{W} \times 100\%$$

Description:

X = Fe concentration obtained from the calibration curve (mg/L)

Y = total solution volume (L)

FP = dilution factor

W = sample weight (g)

### *Determination of Vitamin A Content Using UV-Vis Spectrophotometry*

5 g of the sample was accurately weighed and mixed with 25 mL of *n*-hexane as the extraction solvent. The mixture was thoroughly shaken to facilitate the extraction of carotenoid compounds and then filtered using Whatman filter paper. The filtrate was subsequently passed through anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) to remove any residual moisture. The extraction process was repeated until the sample residue became colorless, indicating complete extraction of carotenoid pigments. The absorbance of the collected filtrate was measured using a UV-Visible spectrophotometer at a wavelength of 450 nm. The vitamin A content was then calculated based on the regression equation obtained from the β-carotene standard calibration curve.

$$\text{Vitamin A (mg/100g)} = \frac{x \cdot Y \cdot fp}{W} \times 100$$

Description:

X = β-carotene concentration obtained from the calibration curve (mg/L)

Y = total volume of the extract solution (L)

FP = dilution factor

W = sample weight (g)

### *Preparation of Iron (Fe) Standard Calibration Curve*

An iron stock solution with a concentration of 1000 ppm was diluted to obtain a 100 ppm working solution in a 25 mL volumetric flask, and the volume was adjusted to the calibration mark with distilled water. Aliquots of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the 100 ppm solution were then transferred into separate 10 mL volumetric flasks. Subsequently, 1 mL of potassium thiocyanate (KSCN) solution and 1 mL of 4 N hydrochloric acid (HCl) were added to each flask. The solutions were diluted to volume with distilled water to produce standard solutions with concentrations of 0, 5, 10, 15, 20, and 25 ppm, respectively. The maximum absorption wavelength was determined using a UV-Visible spectrophotometer. The absorbance of each standard solution was then measured at the selected maximum wavelength. The resulting absorbance values were plotted against the corresponding iron concentrations to construct a calibration curve, and a linear regression equation was generated for the quantitative determination of iron in the cracker samples.

### *Preparation of Vitamin A Standard Calibration Curve*

A 10 mg portion of β-carotene was accurately weighed and dissolved in *N*-hexane to a final volume of 100 mL to prepare the stock solution. Aliquots of 0.25, 0.50, 0.75, 1.00, and 1.25 mL of the stock solution were then transferred into separate volumetric flasks and diluted with *N*-hexane to a final volume of 5 mL, yielding β-carotene standard solutions with concentrations of 5, 10, 15, 20, and 25 mg/L, respectively. The absorbance of each standard solution was measured at a wavelength of 450 nm using a spectrophotometer. The absorbance data

obtained were subsequently used to construct a calibration curve describing the relationship between  $\beta$ -carotene concentration (mg/L) and absorbance.

### Data Analysis

The data obtained in this study were quantitative in nature and were derived from the absorbance measurements of sample solutions using a UV–Visible spectrophotometer. The concentrations of iron (Fe) and vitamin A were calculated using the linear regression equations generated from the respective standard calibration curves. These calculations were used to evaluate the effectiveness of *Moringa oleifera* leaf supplementation in enhancing the iron and vitamin A contents of the cracker samples. The resulting nutrient content data were statistically analyzed using one-way Analysis of Variance (ANOVA) to determine whether significant differences existed among the treatment groups (0%, 5%, 10%, and 15% moringa leaf supplementation). The results were expressed as mean values and presented in the form of tables and graphs to facilitate interpretation and comparison among treatments. If significant differences were detected at the chosen significance level ( $p < 0.05$ ), an appropriate post

hoc multiple comparison test was performed to identify differences between individual treatment means.

## RESULTS AND DISCUSSION

The results of this study demonstrated that the incorporation of *Moringa oleifera* leaf flour was effective in increasing both the iron (Fe) and vitamin A contents of the cracker samples (Table 1). Higher levels of moringa leaf flour supplementation resulted in greater concentrations of these nutrients, indicating the potential of moringa leaves as a natural fortification ingredient for improving the nutritional quality of snack foods (Table 2).

**Table 1.** Iron (Fe) and Vitamin A of *Moringa oleifera* leaf crackers.

Sample	Iron (Fe) (mg/100g)	Vitamin A (mg/100g)
P0 (0%)	0.67	0.00
P1 (5%)	0.53	0.00
	1.10	7.75
P2 (10%)	1.25	7.79
	1.96	14.44
P3 (15%)	2.02	14.40
	3.05	21.78
	2.95	21.89

**Table 2.** One-Way ANOVA for Iron (Fe) and Vitamin A.

		Sum of Squares	df	Mean Square	F	Sig
Iron (Fe)	Between Groups	6.519	3	2.173	312.093	0.000
	Within Groups	0.028	4	0.007		
	Total	6.547	7			
Vitamin A	Between Groups	521.053	3	173.684	90815.292	0.000
	Within Groups	0.008	4	0.002		
	Total	521.060	7			

### Quantitative Determination of Iron (Fe) and Vitamin A Using UV–Vis Spectrophotometry

The quantitative determination of iron (Fe) and vitamin A contents was performed using UV–Visible (UV–Vis) spectrophotometry. The quantitative analysis demonstrated that the treatments applied in this study significantly influenced the concentrations of both iron and vitamin A in the cracker samples. Increasing levels of *Moringa oleifera* leaf flour supplementation resulted in corresponding increases in nutrient content, indicating statistically significant changes among treatments. These findings confirm that the fortification treatment was effective in enhancing the nutritional quality of the product, particularly with respect to iron and vitamin A enrichment.

### Preparation of Standard Calibration Curves for Iron (Fe) and Vitamin A

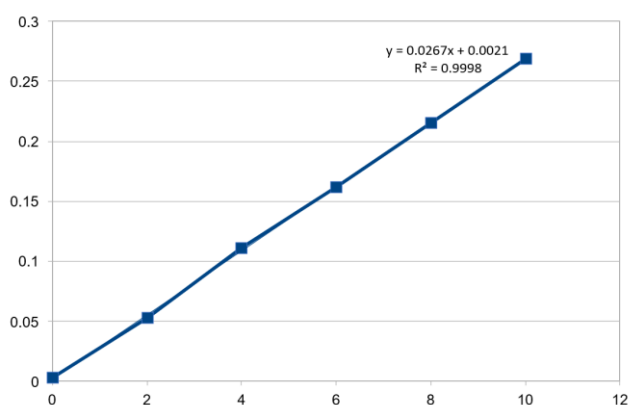
The concentrations of iron (Fe) and vitamin A in the samples were determined using calibration curves generated from a series of standard solutions with known

concentrations. For iron analysis, standard solutions with concentrations of 0, 2, 4, and 6 ppm were prepared, yielding absorbance values of 0.003, 0.053, 0.111, and 0.162, respectively. Absorbance measurements were performed at a wavelength of 480 nm using a UV–Visible spectrophotometer. For vitamin A analysis,  $\beta$ -carotene was used as the standard compound. Standard solutions with concentrations of 2, 4, 6, 8, and 10 mg/L produced absorbance values of 0.198, 0.381, 0.556, 0.713, and 0.892, respectively, measured at a wavelength of 450 nm. The resulting calibration curves exhibited a strong linear relationship between concentration and absorbance, indicating that the measurements were conducted within an appropriate concentration range for quantitative analysis. This linearity confirms the suitability of the analytical method for determining iron and vitamin A contents in the cracker samples. The observed relationship is consistent with the Beer–Lambert Law, which states that absorbance increases proportionally with analyte concentration under constant measurement conditions (Gao et al., 2022).

**Table 3.** Standard calibration data.

Standard Calibration Data	Absorbance (480 nm)
0	0.003
2	0.053
4	0.111
6	0.162

The incorporation of *Moringa oleifera* leaf flour contributed significantly to the enhancement of iron (Fe) content in the cracker products. This observation was supported by the analytical results, which showed that crackers without moringa leaf fortification (0%) exhibited the lowest iron concentration, averaging approximately 0.67 mg/100 g. The addition of 5% moringa leaf flour increased the iron content to approximately 1.10–1.25 mg/100 g, while supplementation at the 10% level resulted in a more substantial increase, reaching approximately 1.96–2.02 mg/100 g. The highest iron concentration was observed in crackers fortified with 15% moringa leaf flour, with values ranging from 2.95 to 3.05 mg/100 g (Figure 2). The progressive increase in iron content indicates a strong positive relationship between the concentration of moringa leaf flour incorporated into the formulation and the resulting iron concentration in the final product. This trend can be attributed to the naturally high iron content of moringa leaves, which serve as an excellent source of dietary iron. Consequently, increasing the proportion of moringa leaf flour in the cracker formulation directly enhanced the mineral content of the product. These findings are consistent with previous studies reporting that *Moringa oleifera* leaves are exceptionally rich in iron and can be effectively utilized as a natural fortification ingredient to improve the nutritional quality of food products (Krisnadi, 2020).

**Figure 2.** Iron (Fe) standard calibration curve.

The linear regression equation was obtained from the standard calibration data, which described the relationship between iron (Fe) concentration and the absorbance values measured by UV–Vis spectrophotometry. The coefficient of determination ( $R^2$ ) reflected the degree of linearity between these two variables (Table 4). The resulting calibration curve

exhibited an upward-sloping straight line, indicating that absorbance increased proportionally with increasing iron concentration. This linear relationship is consistent with the Beer–Lambert Law, which states that absorbance is directly proportional to the concentration of an analyte under constant experimental conditions. Linear regression analysis is widely employed in spectrophotometric studies to establish a quantitative relationship between concentration and absorbance. The resulting regression equation serves as a mathematical model for determining the concentration of an unknown sample based on its measured absorbance value. Therefore, the calibration curve provides an essential basis for accurate and reliable quantification of iron content in food samples. The high degree of linearity obtained in this study confirms the suitability of the UV–Vis spectrophotometric method for iron determination and demonstrates that the analytical procedure complied with the fundamental principles of the Beer–Lambert Law (Putri et al., 2021).

**Table 4.** Standard calibration curve data for  $\beta$ -Carotene and Vitamin A.

$\beta$ -Carotene Concentration (mg/L)	Absorbance (450 nm)
2	0.198
4	0.381
6	0.556
8	0.713
10	0.892

A clear relationship was observed between  $\beta$ -carotene concentration and absorbance values measured at a wavelength of 450 nm. The results demonstrated that increasing concentrations of the standard  $\beta$ -carotene solution produced progressively higher absorbance values. Specifically, the absorbance increased from 0.198 at a concentration of 2 mg/L to 0.892 at 10 mg/L, indicating a consistent positive trend across the concentration range tested (Figure 3). The resulting calibration curve exhibited a strong linear relationship between  $\beta$ -carotene concentration and absorbance, confirming the suitability of the analytical method for quantitative vitamin A determination. This linearity indicates that the spectrophotometric measurements were performed within the optimal working range of the instrument and that the absorbance response was directly proportional to analyte concentration. Such behavior is in accordance with the Beer–Lambert Law, which states that absorbance is directly proportional to the concentration of a substance in solution under constant experimental conditions (Wulandari et al., 2020). The linear regression equation obtained from the calibration curve can therefore be used to accurately determine the concentration of  $\beta$ -carotene in unknown samples based on their absorbance values. Consequently, the standard calibration curve serves as a reliable analytical tool for the quantitative estimation of vitamin A content in *Moringa oleifera* leaf crackers. The high degree of linearity observed further demonstrates the validity and

precision of the UV–Vis spectrophotometric method employed in this study.

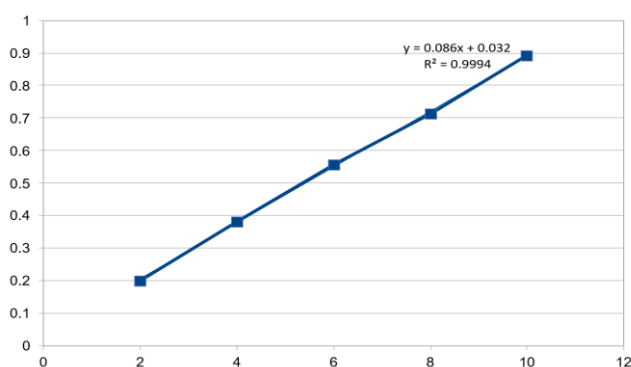


Figure 3. Vitamin A standard calibration curve.

The linear regression equation obtained from the vitamin A standard calibration curve demonstrates the relationship between the concentration of the standard solution and the absorbance values measured. The resulting curve formed an increasing straight line, indicating that higher vitamin A concentrations produced higher absorbance values. This condition is consistent with the Beer–Lambert Law, which states that absorbance is directly proportional to the concentration of a solution. Based on the results of this study, vitamin A content increased with increasing concentrations of leaf flour, although the increase was not always linear because  $\beta$ -carotene, the precursor of vitamin A, is highly sensitive to heat and may undergo degradation during processing. The regression equation obtained was subsequently used to determine the vitamin A content of the samples quantitatively, enabling accurate measurements. (Rahmawati et al., 2021).

## Discussion

The iron and vitamin A contents of the crackers increased as the concentration of *Moringa oleifera* leaf flour increased. In treatment P0 (0%), the iron content ranged from 0.670 to 0.527 mg/100 g, while vitamin A was not detected. Following the addition of 5% moringa leaf flour (P1), the iron content increased to 1.105–1.254 mg/100 g, with vitamin A levels ranging from 7.750 to 7.850 mg/100 g. In treatment P2 (10%), the iron content further increased to 1.963–2.021 mg/100 g, while vitamin A content reached 14.435–14.395 mg/100 g. Meanwhile, treatment P3 (15%) produced the highest values, with iron content ranging from 2.948 to 3.047 mg/100 g and vitamin A content ranging from 21.781 to 21.891 mg/100 g. These results indicate that increasing the concentration of moringa leaf flour led to higher iron and vitamin A contents in the crackers. The enhancement of nutritional value can be attributed to the naturally high concentrations of iron and provitamin A ( $\beta$ -carotene) present in moringa leaves, which contribute significantly to the nutritional composition of food products. Iron plays an essential role in hemoglobin synthesis and

oxygen transport within the body, whereas vitamin A is important for maintaining eye health and strengthening the immune system. The findings of this study are consistent with previous research demonstrating that the incorporation of moringa leaf flour into food products significantly increases iron and  $\beta$ -carotene contents (Zakaria et al., 2020). Similarly, Rahmawati and Adi (2021) reported that moringa leaves possess considerable potential as a food fortification ingredient due to their relatively high iron and vitamin A contents. Therefore, increasing the concentration of moringa leaf flour resulted in improved nutritional quality of the cracker products.

The gradual increase in moringa leaf flour concentration from 0% to 15% also resulted in a corresponding increase in iron (Fe) content. The progressive pattern observed across treatments indicates a strong dose–response relationship, whereby higher levels of moringa leaf supplementation directly enhanced the iron concentration of the crackers. This effect can be explained by the fact that *Moringa oleifera* leaves contain substantial amounts of iron and therefore serve as a major source of this mineral in fortified food systems, as reported by Peñalver et al. (2022) and Trigo et al. (2023). Furthermore, Maharani et al. (2021) stated that the utilization of moringa leaves in food products can significantly improve iron content and overall nutritional quality. Consequently, higher concentrations of moringa leaf flour in the cracker formulation resulted in greater iron enrichment of the final product.

Mechanistically, the increase in iron (Fe) content occurred because the minerals derived from moringa leaves were homogeneously distributed throughout the dough matrix during the mixing process and were largely retained after processing. This explanation is supported by the digestion analysis results, which showed an increase in iron concentration from 0.670 mg/L in the control treatment (P0) to 3.047 mg/L in the highest supplementation treatment, indicating that higher concentrations of moringa leaf flour resulted in greater amounts of dissolved and detectable iron. The thermal stability of iron is an important factor explaining why its concentration did not decrease significantly during processing. As reported by Razzak et al. (2022), minerals are generally resistant to thermal treatments and remain relatively stable under heating conditions. Furthermore, the digestion process performed prior to analysis facilitates the release of iron from the food matrix, allowing metal ions to dissolve completely and be accurately quantified using spectrophotometric methods. The progressive increase in iron content across treatments demonstrates that moringa leaves are an effective source of mineral fortification in cracker products. The stability of iron during processing also highlights the greater resistance of minerals to heat compared with vitamins, which are more susceptible to degradation through thermal and oxidative reactions.

Handayani et al. (2021), who reported that the iron content of moringa-based food products remained stable after processing because minerals possess high resistance to elevated temperatures. Their study also confirmed that the incorporation of moringa leaves significantly enhances the iron content of processed food products.

The non-linear increase in vitamin A content can be explained by the degradation of  $\beta$ -carotene during processing as a result of exposure to heat, oxygen, and light. These factors may cause partial degradation of carotenoid compounds, resulting in final vitamin A concentrations that do not fully reflect the amount initially added to the formulation. This observation is consistent with the findings of Razzak et al. (2022), who reported that carotenoid compounds are highly sensitive to thermal processing and can undergo significant degradation under unfavorable conditions. In addition to nutritional changes, increasing the concentration of moringa leaf flour may also influence the sensory characteristics of the final product. Trigo et al. (2023) reported that higher levels of moringa leaf incorporation can result in darker coloration, a stronger characteristic moringa flavor, and alterations in product texture. These changes indicate a trade-off between nutritional enhancement and consumer acceptability, suggesting that an optimal fortification level should be carefully considered during product development to achieve both improved nutritional quality and favorable sensory properties.

The absorbance values obtained for iron (Fe), ranging from 0.003 to 0.269, demonstrated a linear relationship between iron concentration and light absorption intensity. This finding is consistent with the fundamental principles of spectrophotometry, which state that absorbance is directly proportional to the concentration of an analyte in solution. The spectrophotometric method employed in this study was sufficiently sensitive to detect variations in iron concentration, allowing accurate quantification of the observed increases in iron content, as described by Su et al. (2023). For vitamin A, the observed increase in concentration was also attributable to the addition of moringa leaf flour, which contains high levels of  $\beta$ -carotene, the precursor of vitamin A. Consequently, higher concentrations of moringa leaf flour introduced greater amounts of  $\beta$ -carotene into the food system, as reported by Klimek-Szczykutowicz et al. (2024) and Trigo et al. (2023). However, unlike iron, vitamin A did not always exhibit an increase as consistent or pronounced as that observed for Fe. This difference can be attributed to the instability of  $\beta$ -carotene, which is susceptible to degradation during processing and storage, thereby affecting the final vitamin A content of the product.

The linear regression equations were established after measuring the absorbance values of the iron (Fe) and vitamin A standard solutions using a UV–Visible spectrophotometer. Calibration curves were subsequently constructed based on the relationship between the

concentrations of the standard solutions and their corresponding absorbance values. Data analysis yielded the regression equation  $y = 0.0267x + 0.0021$  for iron and  $y = 0.086x + 0.032$  for vitamin A. The resulting calibration curves demonstrated a linear relationship between concentration and absorbance for both analytes. The coefficients of determination ( $R^2$ ) obtained were 0.9998 for iron and 0.994 for vitamin A, indicating a very strong correlation between concentration and absorbance.  $R^2$  values approaching 1 suggest that most of the variation in absorbance can be explained by changes in analyte concentration. Therefore, the calibration curves generated in this study can be considered valid and suitable for accurate quantitative analysis. In UV–Vis spectrophotometric methods,  $R^2$  values greater than 0.99 are generally regarded as evidence of excellent linearity and indicate that the analytical method is highly reliable for concentration determination (Rahayu et al., 2018).

Variations in the analytical results may also be influenced by factors related to raw materials and processing conditions. Fidyasaria et al. (2024) reported that the nutritional composition of moringa leaves can vary depending on environmental conditions, drying methods, and the form of processing applied to the raw material. The findings of the present study demonstrate a clear cause-and-effect relationship between increasing concentrations of moringa leaf flour and the corresponding increases in iron and vitamin A contents of the crackers. However, this relationship is not determined solely by the amount of moringa leaf flour added. Other factors, including nutrient stability during processing, nutrient bioaccessibility, and interactions with other food components, may also influence the final nutritional composition of the product, as described by Mawouma et al. (2024). Consequently, although the incorporation of moringa leaf flour directly enhances the iron and vitamin A contents of crackers, the effectiveness of this enrichment depends on the stability of bioactive compounds throughout processing, the accessibility of nutrients for absorption, and the balance between nutritional improvement and desirable sensory characteristics (Klimek-Szczykutowicz et al., 2024).

According to the Indonesian Recommended Dietary Allowances (RDA), iron is an essential mineral involved in hemoglobin synthesis and the prevention of anemia, with recommended daily intakes ranging from approximately 8–18 mg per day, depending on age and sex. Meanwhile, vitamin A plays a fundamental role in maintaining visual health, supporting immune function, and promoting cell growth and development, with recommended daily requirements of approximately 500–900  $\mu\text{g}$  Retinol Activity Equivalents (RAE) per day (Ministry of Health of the Republic of Indonesia, 2019). The utilization of nutrient-rich ingredients such as *Moringa oleifera* leaves in cracker production can significantly enhance the levels of these essential micronutrients, thereby contributing to the fulfillment of

daily nutritional requirements and supporting improved public health outcomes (Brar *et al.*, 2022).

## CONCLUSIONS

The addition of *Moringa oleifera* leaf flour effectively increased the iron (Fe) and vitamin A contents of crackers. Higher concentrations of moringa leaf flour resulted in greater nutrient enrichment, with the 15% supplementation treatment producing the highest iron and vitamin A levels. The UV-Vis spectrophotometric method demonstrated excellent linearity and reliability for quantitative analysis, as indicated by the high coefficients of determination ( $R^2 > 0.99$ ). These findings suggest that moringa leaf flour has strong potential as a natural fortification ingredient for developing nutritious crackers and improving the micronutrient content of snack foods.

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**Competing Interests:** The authors declare that there are no competing interests.

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