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## Original Research Article

### Utilization of *Musa Paradisiaca* Pseudostems and *Moringa* Leaves as Fortification for Nutritious Biscuits as a Source of Natural Antioxidants

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#### ABSTRACT

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*Musa paradisiaca* and *Moringa oleifera* are widely cultivated in tropical regions, including Indonesia. *Musa paradisiaca* pseudostems are often discarded after harvest, and *Moringa* leaves are underutilised. *Musa paradisiaca* pseudostem is rich in carbohydrate and crude fibre, while *Moringa* leaves are rich in protein, calcium, phosphorus,  $\beta$ -carotene, and thiamine. This study aimed to determine the nutritional content, antioxidant activity, and quality of *Musa paradisiaca* pseudostem flour (TBP) and *Moringa* leaf flour (TDK) formulated into fortified biscuits. The study measured the carbohydrate, protein, fat, crude fibre, key micronutrients (Ca, P, Fe, Thiamine,  $\beta$ -carotene), moisture, ash content, heavy metal contamination, Total Plate Count (TPC), and antioxidant activity in TBP, TDK, and biscuit formulas (F0, F1, F2, and F3) using appropriate methods. TDK showed the highest protein ( $8.52 \pm 0.007\%$ ) and micronutrient levels, while TBP showed the highest carbohydrate ( $63.46 \pm 0.007\%$ ) and crude fibre ( $2.96 \pm 0.007\%$ ) levels. The combination was effective, with Formula F2 (optimally formulated) demonstrating the best nutritional profile, significantly increasing the combined nutrient and fibre content. Although TBP and TDK flours exceeded the microbial contamination limit, all fortified biscuit formulas (F0, F1, F2, and F3) were safe from microbial ( $< 1.0 \times 10^4$  colony/g) and lead (Pb) heavy metal contamination ( $< 1.0 \times 10^8$  mg/kg). This safety level is attributed mainly to the high-temperature baking process. Both the *Musa paradisiaca* pseudostem and *Moringa* leaves possess valuable compounds suitable for fortification. Formulas F1, F2, and F3 are free of microbial and Pb contamination, making them a promising source of natural antioxidants and essential nutrients.

**Keywords:** *Musa paradisiaca*, Pseudostems, *Moringa*, Leaves, Antioxidant, Nutrition, Fortification, Biscuit

#### Introduction

*Musa paradisiaca* and *moringa* (kelor) are plants that grow readily and are widely distributed in tropical regions such as Indonesia. Both of these plants are utilised as food in Indonesia. The fruit of the *Musa paradisiaca* is frequently used as a food source, while the leaves and fruits of the *Moringa* plant are commonly consumed<sup>1</sup>. *Musa paradisiaca* plants are monocarpic, meaning they only bear fruit once. Consequently, the pseudostem section of the banana plant is typically left as waste after fruit harvest. In South Sulawesi, harvested *Musa paradisiaca* pseudostems are typically discarded or used only as an additive in animal feed. Meanwhile, the utilisation of *Moringa* (kelor) is often limited to vegetables and wound healing, suggesting that *Moringa* leaves are not yet being fully utilised<sup>2</sup>.

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The utilisation of *Musa paradisiaca* pseudostems in food remains uncommon, despite extensive research leveraging other parts of the banana plant (such as the fruit, peel, and blossom) as food ingredients<sup>3,4</sup>. *Musa paradisiaca* pseudostems are rich in dietary fibre, which supports digestion. This pseudostem is reported to aid in preventing diverticulitis, constipation, high cholesterol, colon cancer, obesity, and diabetes mellitus<sup>5</sup>. The cellulose content in banana pseudostems is approximately 63–64%<sup>6</sup>. Furthermore, the starch in banana pseudostems can be used as a substitute for wheat flour. In previous research, banana flour, *Moringa* flour, and tempeh flour were reported to have potential as biscuit ingredients<sup>7</sup>. *Moringa* leaf is a known, easily accessible, nutritious local food source and an excellent antioxidant source<sup>8</sup>. The dried leaves of *Moringa oleifera* contain Moisture 3.0%, Ash 13.5%, fibre 8.5%, Crude lipid 5.0%, Crude protein 5.43%, Carbohydrate 62.57%. The mineral content of the leaves (mg/100 g): Sodium (Na) 0.14, potassium (K) 5.10, Calcium (Ca) 0.28, magnesium (Mg) 0.29, phosphorus (P) 5.58<sup>9</sup>. *Moringa* leaves also contain Vitamins A, B, and C,  $\beta$ -carotene, xanthine, flavonoids, sugars, steroids, alkaloids, coumarins, and fatty acids. They have traditionally been utilised for their therapeutic properties, including anti-hypertensive, anti-obesity, antimicrobial, anti-inflammatory, antioxidant, analgesic, diuretic, anti-cholesterol, and hepatoprotective effects<sup>10</sup>. In this study, a combination of *Musa paradisiaca* pseudostem flour and *Moringa* leaf flour was used to fortify biscuits. This approach aims to make both *Musa paradisiaca* pseudostems and *moringa* leaves more readily consumable, thereby enabling the beneficial properties of these

ingredients to be harnessed for health benefits. The objective of this research was to determine the nutritional content, antioxidant activity, and quality of *Musa paradisiaca* pseudostem flour (TBP) and Moringa leaf flour (TDK) formulated into fortified biscuits. This study is expected to provide an innovative approach to the utilization of *Musa paradisiaca* pseudostems and Moringa leaves as functional food ingredients.

## Materials and Methods

### Materials

The equipment utilised included Atomic Absorption Spectrophotometer 240FS Model Agilent (USA), UV-Vis spectrophotometry Cary 60 Model Agilent (USA), an analytical balance (Shimadzu<sup>®</sup>), a food dehydrator (Wirastar<sup>®</sup>), distillation apparatus (Iwaki<sup>®</sup>), a fume hood (Esco<sup>®</sup>), a furnace (Jisico<sup>®</sup>), an incubator (Memmert<sup>®</sup>), Petri dishes (Anumbr<sup>®</sup>), a Soxhlet extractor (Infitec<sup>®</sup>), a micropipette (OneMed<sup>®</sup>), an oven (Memmert<sup>®</sup>), a water bath (Memmert<sup>®</sup>), and a UV-Vis spectrophotometer (Thermo<sup>®</sup>). Other materials include Pb(NO<sub>3</sub>)<sub>2</sub> (Merck<sup>®</sup>), Fe(NO<sub>3</sub>)<sub>3</sub> (Merck<sup>®</sup>), HNO<sub>3</sub> (Merck<sup>®</sup>), CaCO<sub>3</sub> (Merck<sup>®</sup>), P<sub>2</sub>O<sub>5</sub> (Merck<sup>®</sup>), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Merck<sup>®</sup>), Aquadest (OneLab<sup>®</sup>), Bromthymol Blue indicator (Merck<sup>®</sup>), Thiamine hydrochloride (USP<sup>®</sup>), ethanol, methanol, beta-carotene (Sigma<sup>®</sup>), and Nutrient Agar (Merck<sup>®</sup>).

### Collection and Preparation of Plant Materials

The *Musa paradisiaca* pseudostems and Moringa leaf (kelor) samples were sourced from Japing Village, Pattallassang District, Gowa Regency, South Sulawesi, (5°12'02.3"S 119°32'10.2"E). Both plants were identified at the Botany Laboratory, Department of Biology, Hasanuddin University, and were registered under voucher ID numbers 01979/UN4.11/PT.01.02/2025 and 01980/UN4.11/PT.01.02/2025, respectively. *Musa paradisiaca* pseudostems were collected from plants whose fruits had already been harvested. Specifically, the sample comprised the middle layer, or innermost part, of *Musa paradisiaca* pseudostems. Mature Moringa leaves were harvested, characterised by their dark green colour, starting from the seventh leaflet onward. The Moringa leaves were then separated from their stalks and washed thoroughly. Subsequently, the leaves were dried in an oven at 30 °C until completely dehydrated.

### Preparation of *Musa paradisiaca* Pseudostems Flour (TBP)

*Musa paradisiaca* pseudostems were thoroughly washed and cut into small pieces, approximately 1 cm thick. These pieces were then soaked in a 10% salt solution for 30 minutes. Following soaking, the pseudostem pieces were rinsed with running water, drained, and dried in an oven at 70 °C for 20 hours. The dried *Musa paradisiaca* pseudostems were subsequently pulverised and sifted using a No. 100 mesh sieve.

### Preparation of Moringa Leaf Flour (TDK)

Dried Moringa leaves (1 kg) were pulverised in a blender and then sifted through a No. 100 mesh sieve to make a TDK powder.

### Biscuit Preparation

All ingredients were weighed according to the formulation as shown in Table 1. Powdered sugar, margarine, milk powder, and vanilla were mixed using a mixer until the dough became homogeneous. Once homogeneous, the egg yolks (amount or weight) were incorporated into the dough and mixed for 10 minutes. Then, a sufficient amount of baking soda was added, and the mixture was mixed briefly until homogeneous. Subsequently, wheat flour, *Musa paradisiaca* pseudostems flour, and Moringa leaf flour were added to the mixture and stirred using a spatula until evenly combined. Each batch of dough from the respective formulas (F0, F1, F2, F3) was thinly rolled, shaped into the desired form, placed on a baking sheet, and baked at 125 °C for 15 minutes, until fully cooked.

**Table 1:** Biscuit Formulation for a 200-gram product

Material	F0 (control) (g)	F1 (g)	F2 (g)	F3 (g)
<i>Musa paradisiaca</i> pseudostems flour (TBP)	-	5	10	10
Moringa leaf flour (TDK)	-	10	10	5
Wheat flour	100	85	80	85
Milk powder	20	20	20	20
Powdered sugar	30	30	30	30
Margarine	30	30	30	30
Egg yolk	20	20	20	20
Baking soda	q.s.	q.s.	q.s.	q.s.
Vanilla	q.s.	q.s.	q.s.	q.s.

Notes:

F0 = Biscuit formula with a ratio of wheat flour: *Musa paradisiaca* pseudostems flour (TBP): Moringa leaf flour (TDK) of 100:0:0.

F1 = Biscuit formula with a ratio of wheat flour: *Musa paradisiaca* pseudostems flour (TBP): Moringa leaf flour (TDK) of 85:5:10.

F2 = Biscuit formula with a ratio of wheat flour: *Musa paradisiaca* pseudostems flour (TBP): Moringa leaf flour (TDK) of 80:10:10.

F3 = Biscuit formula with a ratio of wheat flour: *Musa paradisiaca* pseudostems flour (TBP): Moringa leaf flour (TDK) of 85:10:5.

### Quality and quantitative Analysis of *Musa paradisiaca* Pseudostems Flour (TBP), Moringa Leaf Flour (TDK), and Biscuits

#### Lead (Pb) Content

The sample (5 g) was accurately weighed into a porcelain crucible. The crucible was then placed into a furnace until the sample turned white ash. This white ash was dissolved in 5 mL of 6N HCl and heated on an electric heater until dry. It was dissolved in 0.1N HNO<sub>3</sub>, transferred into a 50 mL volumetric flask, and made up to the mark. If necessary, the solution was filtered using ashless filter paper into a polypropylene container. A blank solution was prepared. The absorbance of both the sample and blank solutions was measured using an Atomic Absorption Spectrophotometer (AAS) 240FS Model Agilent (USA) at a wavelength of 283 nm<sup>11</sup>.

#### Total Plate Count (TPC)

A series of dilutions was prepared as needed using sterile Aquadest (distilled water). 1 mL of each sample was pipetted from the 10<sup>-1</sup> to 10<sup>-4</sup> dilution levels into separate sterile Petri dishes in duplicate. Subsequently, 15 mL of molten NA (Nutrient Agar) medium was poured into each Petri dish and homogenised until solidified. All Petri dishes were then inverted and incubated at 35 °C for 48 hours. The growth of colonies on each Petri dish was recorded<sup>12</sup>.

### Nutritional Content Analysis of *Musa paradisiaca* Pseudostems Flour (TBP), Moringa Leaf Flour (TDK), and Biscuits

#### Carbohydrate Content

The carbohydrate content was analysed by difference. This method involves subtracting the sum of the known values of other components (such as moisture, ash, protein, and fat content) from the total 100%<sup>13</sup>.

#### Protein Content

The protein content was analysed using the Kjeldahl method and measured at Labkesmas Makassar I, part of the Indonesian Ministry of Health<sup>13</sup>.

#### Fat Content

The fat content was analysed using the gravimetric method and measured at Labkesmas Makassar I, part of the Indonesian Ministry of Health<sup>13</sup>.

#### Fiber Content

The fibre content was analysed using the gravimetric method and

measured at Labkesmas Makassar I, part of the Indonesian Ministry of Health<sup>14</sup>.

#### Moisture Content

The moisture content was analysed using the gravimetric method and measured at Labkesmas Makassar I, part of the Indonesian Ministry of Health<sup>14</sup>.

#### Ash Content

The ash content was analysed using the incineration method and measured at Labkesmas Makassar I, part of the Indonesian Ministry of Health<sup>13</sup>.

#### Calcium Content

The calcium content was analysed using an Atomic Absorption Spectrophotometer (AAS) 240FS Model Agilent (USA), and measured at Labkesmas Makassar I, part of the Indonesian Ministry of Health<sup>14</sup>.

#### Phosphorus Content

The phosphorus content was analysed using an Atomic Absorption Spectrophotometer (AAS) 240FS Model Agilent (USA), and measured at Labkesmas Makassar I, part of the Indonesian Ministry of Health<sup>14</sup>.

#### Iron Content

The iron content was analysed using an Atomic Absorption Spectrophotometer (AAS) 240FS Model Agilent (USA) and measured at Labkesmas Makassar I, part of the Indonesian Ministry of Health<sup>14</sup>.

#### Beta-Carotene Content

The  $\beta$ -carotene content was analysed using a UV-Vis spectrophotometer (Cary 60 Model, Agilent, USA) and measured at Labkesmas Makassar I, Indonesian Ministry of Health<sup>15</sup>.

#### Thiamine Content

The thiamine content was analysed using a UV-Vis spectrophotometer, Cary 60 Model Agilent (USA) at the Poltekkes Makassar, Indonesian Ministry of Health<sup>15</sup>.

#### Sample Treatment

The sample was homogenised, and 5 g was accurately weighed using an analytical balance. It was placed into a 50 mL Erlenmeyer flask and made up to the mark with Aquadest (distilled water). The solution was homogenised using a magnetic stirrer for 60 minutes, then filtered through filter paper into a 50 mL volumetric flask. The volume was brought up to the mark with aquadest and allowed to stand for 1 hour.

#### Preparation of Thiamine Stock Solution

About 25 mg of thiamine was weighed and dissolved in a 50 mL volumetric flask. Aquadest was added to the mark to obtain a thiamine stock solution at 500 ppm.

#### Determination of Maximum Wavelength

A 100 ppm thiamine solution was prepared by pipetting 5 mL of the stock solution into a 25 mL volumetric flask. Then, 1.5 mL of ammonia buffer, 3 mL of 0.05% bromothymol blue, and 1 mL of 1% polyvinyl alcohol were added, and the volume was brought up to the mark with Aquadest. A further dilution to 30 ppm was prepared by pipetting 3.75 mL into a 10 mL volumetric flask and filling it to the mark. The maximum wavelength ( $\lambda_{\text{max}}$ ) was measured using a UV-Vis spectrophotometer, yielding 616 nm.

#### Preparation of the Calibration Curve

A series of standard solutions was prepared from the thiamine stock solution with concentrations of 10, 15, 20, 25, and 30 ppm. The absorbance of each solution was measured at the thiamine maximum wavelength using a UV-Vis spectrophotometer, and subsequently, the thiamine calibration curve was constructed.

#### Determination of Thiamine Content in the Sample

The thiamine content in the sample was determined by pipetting 5 mL

of the sample filtrate into a 25 mL volumetric flask. 1.5 mL of ammonia buffer, 3 mL of 0.05% bromothymol blue, and 1 mL of 1% polyvinyl alcohol were added. The volume was then brought up to the mark with Aquadest, and the solution was homogenised. The absorbance was measured using a visible spectrophotometer at the thiamine maximum absorbance wavelength (616 nm). Finally, the thiamine content in the sample was determined using the regression equation from the calibration curve.

#### Determination of Antioxidant Activity of *Musa paradisiaca pseudostems* flour (TBP), *Moringa* leaf flour (TDK), and Biscuit Products<sup>7</sup>

The antioxidant activity of the samples was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. In this assay, a 0.02 g sample was dissolved in 20 mL of methanol. The solution was then homogenised using a magnetic stirrer for 15 minutes. Subsequently, the stock solution was diluted to 500 ppm. The 500 ppm solution was further diluted to concentrations of 400 ppm, 300 ppm, 200 ppm, and 100 ppm. Subsequently, 2 mL of each concentration was pipetted using a micropipette and placed into a test tube. 2 mL of DPPH reagent was then added, and the mixture was vortexed until homogeneous. The solutions were then incubated in a dark room for 30 minutes. After 30 minutes, the antioxidant activity was determined by measuring absorbance at 517 nm using a UV-Vis spectrophotometer (Cary 60, Agilent, USA).

#### Data Analysis

The data obtained were analysed statistically using ANOVA with post hoc Tukey's on GraphPad Prism 10.

#### Results and Discussion

*Musa paradisiaca* pseudostems (TBP) are part of the plant rarely consumed by the community in South Sulawesi. However, this plant segment contains numerous compounds beneficial to health. The same applies to the *Moringa* (kelor) plant. *Moringa* leaves contain bioactive compounds with significant antioxidant properties, making them highly valuable for health. Nevertheless, in South Sulawesi, *Moringa* is predominantly used as a vegetable, limiting consumption of its leaves among the local population, particularly children. The results of this study showed the contents of TBP, *Moringa* leaf flour (TDK), and the fortified biscuit formulas (Table 2). Additionally, the results of the qualitative analysis of lead (Pb) contamination and microbial load in *Musa paradisiaca* pseudostem flour (TBP) and *Moringa* leaf flour (TDK), as well as in the fortified biscuit formulas, are presented in Table 3. The results of the antioxidant activity testing of *Musa paradisiaca* pseudostems flour (TBP), *Moringa* leaf flour (TDK), and the fortified biscuit formulas are presented in Figure 2. The data from this study show that *Musa paradisiaca* pseudostem flour (TBP) contains the highest amount of carbohydrates and crude fibre among the biscuit formulas, followed by *Moringa* leaf flour (TDK) and the other biscuit formulas. Cellulose and hemicellulose are fibrous constituents found in banana stems and other plants. The cellulose content in *Musa paradisiaca* pseudostems is approximately 74.37%<sup>16</sup>. Conversely, *Moringa* leaf flour (TDK) contains higher amounts of protein, calcium, phosphorus, and  $\beta$ -carotene than *Musa paradisiaca* pseudostem flour (TBP), F0, F1, F2, and F3. The nutritional values of the individual flours are expected to be mutually complementary when combined to form biscuits. However, it is undeniable that the heating during baking can affect the concentration of each nutrient. Crude fibre contains 50–80% of total cellulose, 10–15% of lignin, and only 20% of hemicellulose<sup>17</sup>. Crude fibre is a type of insoluble fibre with vital functions in the human diet due to its capacity to support digestive health and prevent various chronic diseases. Crude fibre, primarily composed of lignin and cellulose, is indigestible by human enzymes and thus reaches the large intestine intact. In the colon, this fibre helps increase faecal bulk, facilitates bowel movements, and contributes to a healthy gut microbiota<sup>18</sup>. According to the WHO, the recommended daily fibre intake for adults is > 25 g/day.

**Table 2:** Mean Content of Compounds in *Musa paradisiaca* Pseudostem Flour (TBP), Moringa Leaf Flour (TDK), and Fortified Biscuit Formulas

Sample	Carbohydrate (%)	Protein (%)	Fat (%)	Crude fiber (%)	Moisture content (%)	Ash content (%)	Calcium (µg/g)	Phosphorus (%)	Iron (µg/g)	β-carotene (µg/g)	Thiamine (mg/g)
<i>Musa paradisiaca</i> Pseudostems Flour (TBP)	89.07 ± 0.007	0.85 ± 0.007	0.42 ± 0.007	9.93 ± 0.007	0.103 ± 0.031	9.75 ± 0.007	5966.45 ± 4.738	0.355 ± 0.007	0.0633	26.29 ± 0.707	79.14 ± 1.233
Moringa Leaf Flour (TDK)	63.46 ± 0.007	22.58 ± 0.007	2.24 ± 0.007	2.96 ± 0.007	0.008 ± 0.004	11.735 ± 0.007	15417.2 ± 11.172	0.625 ± 0.007	0.0452	3171.82 ± 0.707	83.66 ± 0
F0	73.85 ± 0.007	8.52 ± 0.007	15.99 ± 0.007	0.39 ± 0.007	0.067 ± 0.003	1.325 ± 0.007	119.5 ± 22.769	0.205 ± 0.007	0.0682	32.59 ± 0.707	81.86 ± 1.801
F1	75.61 ± 0.007	8.15 ± 0.007	14.23 ± 0.007	0.44 ± 0.007	0.059 ± 0.001	2.065 ± 0.007	1608.35 ± 2.051	0.265 ± 0.007	0.0641	1221.54 ± 0.707	83.63 ± 0.009
F2	70.91 ± 0.007	7.99 ± 0.007	18.55 ± 0.007	0.78 ± 0.007	0.073 ± 0.003	2.59 ± 0.007	1854 ± 2.263	0.375 ± 0.007	0.0716	1431.86 ± 0.707	83.66 ± 0
F3	71.60 ± 0.007	7.52 ± 0.007	16.99 ± 0.007	0.46 ± 0.007	0.067 ± 0.003	2.12 ± 0.007	1136.2 ± 0.226	0.225 ± 0.007	0.0691	2567.96 ± 0.707	83.65 ± 0.009

Dietary fibre is known to reduce the risk of several chronic conditions, such as obesity, hypertension, diabetes, cardiovascular diseases, and cancer. However, fibre consumption remains relatively low in both high- and middle-income countries<sup>19</sup>. Fibre is a vital component of the human diet for both children and adults to maintain health. It serves as an energy source for gut microorganisms. The fermentation of fibre by gut microorganisms produces various compounds that are beneficial for both short- and long-term health and can boost the immune system and help prevent allergic, inflammatory, and digestive disorders. It has been shown to reduce the risk of obesity, diabetes, hypertension, and coronary heart disease. Fibre deficiency can lead to several disorders, including constipation, irritable bowel syndrome, and immune-related disorders<sup>17,20,21</sup>. In the fortified biscuit products, the amount of crude fibre contained in formulas F0, F1, F2, and F3 is much lower than the crude fibre content found in the raw *Musa paradisiaca* pseudostem (TBP) and Moringa leaf flours (TDK). This may be due to the limited incorporation of *Musa paradisiaca* pseudostem flour (TBP) and Moringa leaf flours (TDK), which range from 5% to 10% in the biscuit formulas, and the heating process could also decrease dietary fibre content as the moisture content decreases<sup>22</sup>. A closer look using Tukey's Test in Figure 1d reveals that only the comparison between F1 and F3 shows a non-significant (ns) difference ( $p > 0.05$ ). In contrast, the differences among the other treatments are significant ( $p \leq 0.01$ ). The data showed that Moringa leaf flour (TDK) exhibited the highest calcium content compared to *Musa paradisiaca* pseudostem flour (TBP) and all fortified biscuit formulas (F0, F1, F2, and F3). Among the fortified biscuit formulas, F2 had the highest levels of calcium, iron, and phosphorus, followed by biscuits F1, F3,

and F0. Tukey's Test (Figures 1g and 1i) showed that the differences in calcium and iron content between treatments were highly significant ( $p \leq 0.01$ ). In Figure 1h, the phosphorus content across all treatments also appeared highly significant, with a  $p$ -value  $\leq 0.01$ , except for the comparison between the phosphorus content of *Musa paradisiaca* pseudostem flour (TBP) and F2, and the comparison between F0 and F3, with a  $p$ -value  $> 0.05$  (Figure 1h). Calcium and phosphorus are crucial for bone formation, especially in children, while iron is necessary for red blood cells to deliver oxygen optimally throughout the body. Children born to iron-deficient mothers also exhibit natural impairments in learning and memory that can persist into adulthood<sup>23,24</sup>. Iron (Fe) is primarily absorbed in the duodenum and the upper jejunum. Iron in food exists in two forms: heme iron (HI) and non-heme iron (NHI). Both HI and NHI likely form a highly dynamic pool of cytosolic iron after absorption<sup>25</sup>. Calcium, on the other hand, is the most abundant mineral in the human body, followed by phosphorus. Insufficient calcium intake can lead to high blood pressure, whereas excessive calcium intake may reduce iron absorption. Phosphorus, in addition to its role in bone formation, also assists in digestion and the elimination of metabolic waste<sup>26</sup>. Results of this study (Table 3) show that Moringa leaf flours have lead (Pb) content exceeding the allowable limit of 0.5 mg/kg<sup>27</sup>. Lead can enter the human body through food and drink, inhalation of Pb-contaminated air, and through skin and eye contact. Lead (Pb) in food can originate from raw materials and other auxiliary ingredients, during processing, and during storage<sup>28</sup>. Heavy metals can also enter the environment through natural processes (such as rock weathering and soil erosion) and human activities (such as mining, indiscriminate disposal of industrial waste, uncontrolled use of pesticides, and others)<sup>29</sup>.

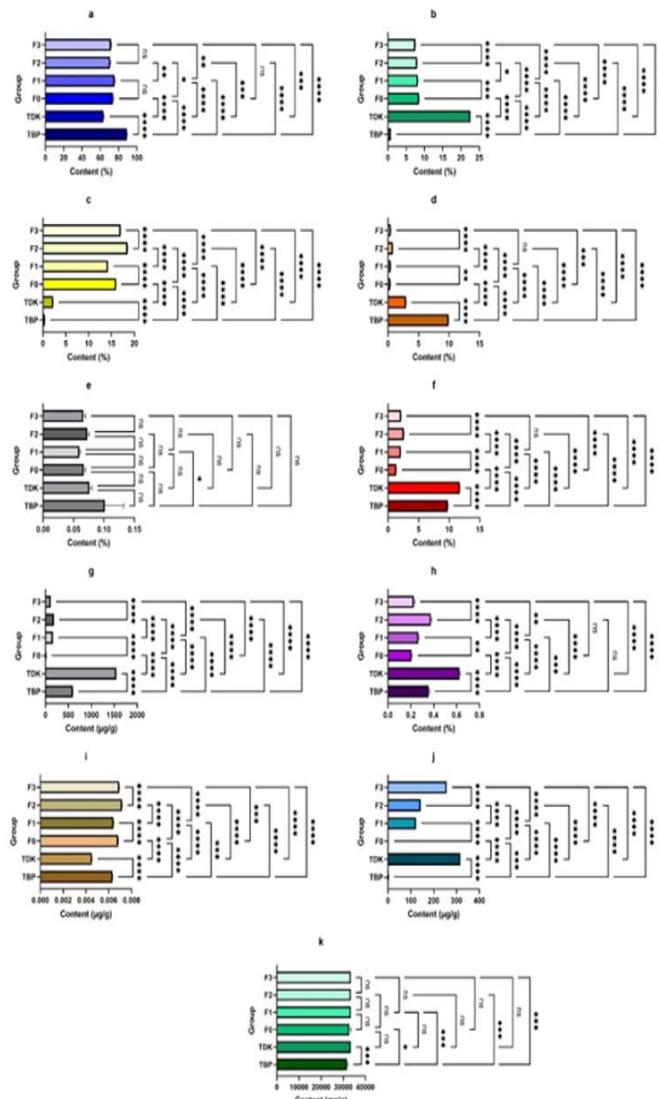
The metal content in plants depends on the type of metal, the soil's chemical elements, soil pH, and the granularity (texture) of the soil in which they grow. Subsequently, these metals will accumulate in the plant's roots and leaves<sup>30</sup>. Lead (Pb) is actively absorbed and can passively diffuse across the intestinal wall. Following absorption, lead is distributed to the body's organs. Lead in the bloodstream can reduce haemoglobin levels. Heme is synthesised from coenzyme A (CoA) and glycine with pyridoxal as a cofactor, subsequently combining with iron to form haemoglobin. ALAD (Aminolevulinic acid dehydratase) and heme synthase are enzymes highly susceptible to lead during heme formation. The active groups of the ALAD enzyme will bind to the synthesised lead metal present in the body, halting the hemoglobin formation reaction and shortening the lifespan of red blood cells<sup>31</sup>. Lead can cause damage to human organs, particularly the nervous, cardiovascular, reproductive, and hematopoietic (blood-forming) systems<sup>32,33</sup>. Malnutrition exacerbates the rise in blood lead levels. A low-calcium diet will increase lead levels in soft tissues and cause toxic effects on the hematopoietic system. Deficiencies in calcium and phosphorus will also increase intestinal lead absorption. Iron deficiency, low-protein diets, and high-fat diets are also known to enhance lead uptake. In bone, lead is found in the form of Pb-phosphate (Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>), and as long as lead remains bound within the bone, it does not cause pain symptoms in the affected individual. However, the danger arises from lead toxicity caused by impaired calcium absorption, which desorbs calcium from bone and releases lead deposits. A diet low in phosphate will similarly trigger the release of lead from the bone into the bloodstream<sup>34</sup>. Lead exposure can cause a decline in the quality of male semen fluid. If the concentration of lead in the blood exceeds 20 µg/dL, it can increase the risk of anaemia and reduce haemoglobin levels<sup>35,36</sup>. Unintentional consumption of lead by children can negatively affect their nutritional status, consequently impacting their growth and development<sup>35</sup>.

**Table 3:** Content of Lead Contamination (Pb) and Total Plate Count (TPC) in *Musa paradisiaca* Pseudostem flour (TBP), Moringa Leaf Flour (TDK), and Fortified Biscuit Formulas

Sample	Lead (Pb) (mg/kg)	Total Plate Count (TPC) (colony/g)
<i>Musa paradisiaca</i>	2.844 x 10 <sup>-7</sup>	2.1 x 10 <sup>4</sup>
Pseudostems Flour (TBP)		
Moringa Leaf Flour (TDK)	7.853 x 10 <sup>-7</sup>	5.5 x 10 <sup>4</sup>
F0	< 1 x 10 <sup>-8</sup>	2.2 x 10 <sup>3</sup>
F1	< 1 x 10 <sup>-8</sup>	3.8 x 10 <sup>3</sup>
F2	< 1 x 10 <sup>-8</sup>	8.5 x 10 <sup>2</sup>
F3	< 1 x 10 <sup>-8</sup>	4.6 x 10 <sup>3</sup>
References <sup>54,63</sup>	Max. mg/kg	Max. 1 x 10 <sup>4</sup> colony/g
	0.2	

This study also included the analysis of three macronutrients—carbohydrate, protein, and fat—contained in *Musa paradisiaca* pseudostem flour (TBP), Moringa leaf flour (TDK), and the fortified biscuit formulas (F0, F1, F2, and F3). The carbohydrate content is measured using the by-difference method, which involves subtracting the total percentages of other components (protein, fat, moisture, and ash) from 100%. The required portions of carbohydrate, protein, and fat must be considered for maintaining health, especially in the prevention of obesity-related diseases and other degenerative diseases, such as cardiovascular disease and hypertension<sup>36,37</sup>. Daily carbohydrate intake of < 10% or 20 – 50 g is considered very low-carbohydrate, < 26% or < 130 g is considered low-carbohydrate, 26 – 44% is considered moderate-carbohydrate, and ≥ 45% is considered high-carbohydrate intake<sup>36</sup>. The carbohydrate content in *Musa paradisiaca* pseudostem flour (TBP) is higher than in Moringa leaf flour (TDK). *Musa paradisiaca* pseudostem contains high levels of starch and cellulose, estimated at around 93.36 ± 0.74%<sup>38,39</sup>. Hydrolysis under acidic conditions converts cellulose into D-glucose. The carbohydrate content in *Musa paradisiaca* pseudostem flour (TBP) relative to Moringa leaf

flour (TDK) and the fortified biscuit formulas (F0, F1, F2, and F3) was highly significant based on the Tukey's Test shown in Figure 1a, with a p-value ≤ 0.01. Similarly, the carbohydrate content in Moringa leaf flour (TDK) relative to the fortified biscuit formulas was highly significant. Among the fortified biscuit formulas, F0 showed a significant difference in carbohydrate content compared to F2 (p-value ≤ 0.05), while the difference between F0 versus F1 and F3 was non-significant (ns) (p-value > 0.05). The carbohydrate content of F1 differed significantly from that of F2 and F3. The F1 formula had the highest carbohydrate content compared to F2, F3, and F0. The lowest carbohydrate content among the fortified biscuit formulas was found in F2. Another macronutrient found in *Musa paradisiaca* pseudostem flour (TBP) is protein. Protein is composed of amino acids (C, H, O, N)<sup>40</sup>. In this study, the Kjeldahl method was used to measure protein content, with protein levels quantified from total nitrogen in the tested food. Nitrogen is the primary element in protein composition, accounting for approximately 16% of the total protein<sup>41</sup>. Moringa leaf flour (TDK) showed the highest protein content in Table 2 compared to *Musa paradisiaca* pseudostem flour (TBP) and the biscuit formulas F0, F1, F2, and F3. The protein content across all treatments showed a highly significant difference (p ≤ 0.01; Figure 1b). This aligns with previous research indicating that Moringa leaves contain about 21–33% protein<sup>42</sup>. Previous studies have established that Moringa leaves are a source of protein, essential amino acids, vitamins, and minerals, and possess antioxidant activity due to the presence of flavonoids and other phenolic compounds<sup>43,44,45</sup>. Moringa leaf extract has also demonstrated antidiabetic activity in human trials by increasing catalase (CAT) and malondialdehyde (MDA) levels, reducing fasting plasma glucose, haemoglobin, low-density lipoprotein cholesterol, and very-low-density lipoprotein cholesterol, and increasing insulin levels in healthy individuals<sup>46</sup>. Furthermore, it possesses therapeutic properties related to immune system function, which requires adequate intake of energy, protein, and micronutrients such as iron, copper, and vitamins that facilitate intercellular communication during an immune response<sup>45</sup>. In this study, the fat content of Moringa leaf flour (TDK) was higher than that of *Musa paradisiaca* pseudostem flour (TBP). As shown in Figure 1c, Tukey's Test revealed that fat content differed significantly across all treatments (p-values ≤ 0.01). The F2 fortified biscuit formula had a higher fat content than its raw ingredients (*Musa paradisiaca* pseudostem flour (TBP) and Moringa leaf flour (TDK)). This finding may be attributed to the inclusion of margarine in the biscuit formulation. Margarine is a semi-solid fat product that contains not less than 80% fat<sup>47</sup>. Fat is fundamentally essential for the body and plays a role in children's growth and development. Fat functions as an energy source, facilitates the absorption of fat-soluble vitamins (A, D, E, K), and is crucial for hormone and brain development<sup>48,49</sup>. The moisture content analysis in this study aimed to determine the percentage of water present in *Musa paradisiaca* pseudostem flour (TBP), Moringa leaf flour (TDK), and the fortified biscuit formulas (F0, F1, F2, and F3). The presence of water in a material can affect its shelf life and microbial contamination<sup>22, 50</sup>. During the processing of raw materials such as *Musa paradisiaca* pseudostem flour (TBP) and Moringa leaf flour (TDK), the drying process reduces moisture content, resulting in a lower mass. This reduction inhibits the rate of chemical reactions and microbial growth, thereby increasing the material's shelf life. Similarly, the baking process for the fortified biscuit products F0, F1, F2, and F3 can reduce their moisture content. Moisture content in food products can affect their appearance, texture, and taste<sup>22, 50</sup>. In Table 2, all samples, namely *Musa paradisiaca* pseudostem flour (TBP), Moringa leaf flour (TDK), and the fortified biscuit formulas (F0, F1, F2, and F3), had a moisture content below the maximum limit of 5% required by the SNI (Indonesian National Standard)<sup>51</sup>. The Tukey's Test in Figure 1e showed a significant difference in moisture content between *Musa paradisiaca* pseudostem flour (TBP) and F1 (p-value ≤ 0.01). The ash content analysis aims to detect the presence of inorganic mineral residue in a sample, whether it is a raw material or a processed product. Ash is the inorganic mineral residue left after organic matter has undergone complete combustion. Food products may contain a maximum ash content of 12%, while fresh materials may contain a maximum of 5%.



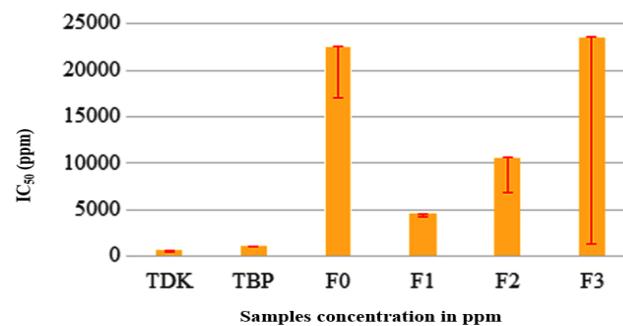
**Figure 1:** Tukey's Test Chart for Compound Content in *Musa paradisiaca* pseudostem flour (TBP), Moringa leaf flour (TDK), and Fortified Biscuit Formulas (F0, F1, F2, F3).

Information: a = carbohydrate; b = protein; c = fat; d = crude fibre; e = moisture content; f = ash content; g = calcium; h = phosphorus; i = iron; j =  $\beta$ -carotene; k = thiamine; ns = non-significant with p-value > 0.05; \* = significant with P-value  $\leq 0.05$ ; \*\*, \*\*\*, \*\*\*\* = very significant with P-value  $\leq 0.01$ .

The minerals frequently found in ash include calcium, potassium, iron, zinc, and other metals<sup>52</sup>. In Table 2, *Musa paradisiaca* pseudostem flour (TBP) and Moringa leaf flour (TDK) showed relatively higher ash content than the fortified biscuit formulas (F0, F1, F2, and F3). Tukey's Test for ash content, as shown in Figure 1f, indicated that all treatments differed significantly from one another, except for the comparison between F1 and F3, which was not significant ( $p > 0.05$ ). In addition to heavy metal testing, microbial testing is essential for assessing the quality of raw materials and final products. The Total Plate Count (TPC) indicates the number of growing microorganisms in a sample. This aims to establish the product's hygiene and quality. If microbial contamination occurs in a product, especially food, it can lead to food poisoning and potentially cause illnesses such as diarrhoea. Microorganisms can contaminate food materials during processing, through equipment used in production, and from the air. The presence of pathogenic organisms can lead to changes in product quality<sup>12,53</sup>.

Both *Musa paradisiaca* pseudostem flour (TBP) and Moringa leaf flour (TDK) have a Total Plate Count (TPC) that exceeds the maximum permitted limit of  $1 \times 10^4$  colony/g<sup>54</sup>. Conversely, the fortified biscuit formulas (F0, F1, F2, and F3) have values below the allowable limit. The microbial growth in biscuit products F0, F1, F2, and F3 is likely inhibited due to the biscuit manufacturing process, specifically the high-temperature heating (baking) at 120 °C. This heating process also reduces the moisture content of the products, thereby making it difficult for microbial cells to proliferate<sup>55</sup>.

In the antioxidant activity test, *Musa paradisiaca* pseudostem flour (TBP) showed lower antioxidant activity than Moringa leaf flour (TDK). This may be due to the lower concentration of antioxidant compounds in the *Musa paradisiaca* pseudostem compared to those in the Moringa leaf. The antioxidant activity of Moringa leaf showed an  $IC_{50}$  of  $622.10 \pm 58.41$  ppm. This result aligns with previous studies reporting that the methanol extract of Moringa leaves had a  $IC_{50}$  of approximately 720 ppm in the DPPH assay<sup>43</sup>. The antioxidant activity of the fortified biscuits (F0, F1, F2, and F3) was also considered very low, with  $IC_{50}$  values  $> 200$  ppm (Figure 2)<sup>56</sup>. The biscuits' antioxidant activity was reduced, likely due to the high-temperature baking process, which may have degraded antioxidant compounds. This finding aligns with previous research showing that prolonged high-temperature heating leads to the decomposition of antioxidant compounds, thereby diminishing their antioxidant capacity.



**Figure 2:** Antioxidant Activity of *Musa paradisiaca* Pseudostems Flour (TBP), Moringa Leaf Flour (TDK), and Fortified Biscuit Formulas

In this study, Moringa leaf flour (TDK) showed higher levels of beta-carotene and thiamine than *Musa paradisiaca* pseudostem flour (TBP).  $\beta$ -carotene and thiamine possess natural antioxidant activity. Thiamine functions as a coenzyme in carbohydrate, protein, and fat metabolism and also acts as a ROS (Reactive Oxygen Species) scavenger that can eliminate hydroxyl radicals ( $HO\cdot$ ) more effectively than hydroperoxyl radicals ( $HOO\cdot$ )<sup>57,58,59</sup>.  $\beta$ -carotene functions in maintaining plasma membrane integrity, cell differentiation, embryonic development, and immune and gastrointestinal function<sup>60</sup>. Furthermore,  $\beta$ -carotene is essential as an antioxidant against peroxides in cells and tissues<sup>61</sup>. Beta-carotene can terminate free radical reaction chains and inhibit lipid peroxidation in liposomes<sup>62</sup>. In Tukey's Test for thiamine content, *Musa paradisiaca* pseudostem flour (TBP) showed a highly significant difference from all other treatments (TDK, F0, F1, F2, and F3), with p-values  $\leq 0.01$  (Figure 1k). Table 2 shows that Moringa leaf flour (TDK) and the F2 fortified biscuit formula have high thiamine levels. The Tukey's Test for  $\beta$ -carotene content showed that all samples (TBP, TDK, F0, F1, F2, and F3) had a highly significant difference from one another (Figure 1j), with a p-value  $\leq 0.01$ . In Table 2, the highest  $\beta$ -carotene content was found in Moringa leaf flour (TDK), and the lowest was in *Musa paradisiaca* stem flour (TBP).

## Conclusion

*Musa paradisiaca* pseudostem and Moringa leaves contain crude fibre, macronutrients (carbohydrate, protein, and fat), and micronutrients (calcium, phosphorus, iron,  $\beta$ -carotene, and thiamine) that can be used for the fortification of nutritious biscuits and as a source of natural

antioxidants. The fortified biscuit formulations F1, F2, and F3 were free of microbial and heavy metal contamination, including lead (Pb), and met the required quality standards for biscuit products.

### Conflict of Interest

The authors declare no conflict of interest.

### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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